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THE AMERICAN PHYSIOLOGICAL SOCIETY

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VOL. 15, No 1

*Issued January, 1935*

BALTIMORE, U. S. A.

Entered as second-class matter, January 15, 1921, at the Post Office at Baltimore., Md. under the act of March 8, 1879

*Made in United States of America*

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$6.00 per volume in the United States, net postpaid
$6.25 per volume in Canada, net postpaid
$6.50 per volume elsewhere, net postpaid
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PHYSIOLOGICAL REVIEWS

VOL. 15 JANUARY, 1935 No. 1

# PHYSIOLOGY OF THE STEROLS, INCLUDING VITAMIN D

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The sterols are complex hydroaromatic secondary alcohols which occur widely, if not abundantly, in living matter. The familiar example is cholesterol, known since the eighteenth century as the principal component of gallstones. Cholesterol forms glistening crystals with characteristic notched corners, by means of which it was early recognized in many morbid structures of the human body. Later it was found to be a constituent of normal tissues. The brain contains an ounce of it, and apparently every organ contains it in appreciable amount.

Vegetable sterols were at first confused with cholesterol. Later they were known as plant cholesterols or phytosterol. The all-inclusive term, sterol, which came into use about 1911, now comprises a family of considerable size. It is usually restricted to those crystalline alcohols found in the unsaponifiable fractions of oils and fats, but not of waxes.

There are groups of physiologically important substances which are fundamentally sterols in structure, although they are generally classified according to their function. Among these are the bile acids and certain cardiac poisons, sex hormones, venoms, and vitamins.

In a short space one can mention merely a fraction of the great number of investigations published on the sterols. It will be necessary in some instances to cite other reviews, and frequently to omit investigations which are mainly of clinical or chemical significance.

## DISTRIBUTION OF STEROLS IN THE ANIMAL AND VEGETABLE KINGDOMS.

*Discovery of cholesterol.* The study of sterols began with investigations on the dissolution of gallstones. Although gallstones have been known, since the time of Vallisneri (1733), to be soluble in alcohol and in turpentine, the isolation of their main constituent from solution was first carried out by Poulletier de la Salle, *circa* 1769.

Poulletier, the author of the French edition of the London Pharmacopœia, is said to have undertaken to ascertain the nature of gallstones by

determining their solubility in alcohol. In this research, which came of a suggestion from Sénac, he observed that the alcoholic solution deposited brilliant leaflets of a substance somewhat like boric or benzoic acid in appearance. Poulletier communicated his observations to Macquer, who mentioned them in the Dictionnaire de Chymie (1788), and to de Fourcroy, who gave a more detailed account of them (1789) after the death of the discoverer. It is uncertain whether Poulletier himself published a report of the experiments; he probably did so, for Dietrich (1788) was acquainted with his findings.

Poulletier's preparation was repeated by Conradi (1775): ". . . . so when the spirit was cooled the flaky coagulum, white, transparent, exceedingly shiny and very light, very similar to flowers of boric acid in appearance, adhered to the bottom of the glass." Conradi added the observation that the substance of gallstones is soluble in ether and crystallizes out of this solvent, as well as from alcohol.

Similar experiments were carried out by Delius (1782), and also by Dietrich (1788), who, working under the guidance of Professor Gren, again prepared the scaly substance by digesting gallstones with alcohol, and gave the first analysis of gallstones:

| | |
|---|---|
| Waxy material | 85 per cent |
| Gluey material | 15 per cent |

Gren (1789) published the chemical sections of Dietrich's Latin thesis in German.

de Fourcroy (1789) regarded the waxy, scaly substance as a variety of spermaceti and adipocere, but Chevreul (1815) distinguished it from these by showing that it was unsaponifiable. Chevreul (1816) named it cholesterine (*chole*, bile; *stereos*, solid).

*Cholesterol in man and the higher animals—early researches.* Soon after cholesterol was discovered, its occurrence in man and the higher animals was investigated extensively. As early as 1853 Robin and Verdeil were able to cite more than 100 references to it, mostly in a pathological connection. The more important contributions were summarized by Flint (1862) as follows (reference dates added): ". . . . Chevreul (1815) established the difference between the adipocire and the cholesterine, giving a full description of the cholesterine. He extracted it from the bile (1824) of the human subject, of the bear, and of the pig. After that time a number of chemists found it in the gall-stones and intestinal concretions. Lassaigne (1818, 1825, 1836) found it in a cerebral tumour, Guérard (1825) in hydatid cysts of the liver, Morin

(1825) in the liquid from an abdominal tumour, Caventou (1825) in the matter from an abscess under the malar bone, and a number of others in tumours in various situations. In 1830 it was discovered in the blood by Denis, and afterwards described by Boudet, who wrote an elaborate article on the composition of the serum of the blood in 1833, in which he describes the cholesterine and a new substance which he called *seroline*.[1] It was also detected in normal blood by Lecanu (1838) and Marchand (1839, 1844). Couerbe (1834), who made elaborate researches into the chemical composition of the cerebral substance, pointed out the existence of cholesterine in the brain. Lebert (1845) found it in the substance of cancerous tumours, Curling (1843) found it in the fluid of hydrocele, Simon (1846) extracted it from the meconium, and Preuss (1835) discovered it in the substance of crude tubercle. Of late authors, Becquerel and Rodier (1844a, b, 1854) have been most extended in their investigations of this principle. They have made a number of careful quantitative analyses of the blood for this substance in health and disease."

More briefly Berthelot (1859) summarized the state of knowledge by describing cholesterol as a substance discovered in gallstones "et retrouvée depuis dans la bile, dans le cerveau, et dans la plupart des liquides normaux ou pathologiques de l'économie humaine . . . ." He prepared esters of cholesterine, thereby establishing its alcoholic nature, which in English and French usage of today is indicated by the termination, -ol, thus, cholesterol. He anticipated the discovery of the existence of cholesterol esters in the body, and of the relation of cholesterol to the bile acids.

Lecanu (1829) found cholesterol in the yolk of egg. Gobley (1846) and Menozzi (1908) showed egg cholesterol to be identical with that from gallstones and bile. Gobley was the discoverer of lecithin (1846, 1847, 1850); he noted the frequent association of cholesterol and lecithin in such diverse situations as the eggs of hens (1846), the roe of carp (1850), the milt of carp (1851a; *cf.* Miescher, 1874, on salmon), human blood (1852), and bile (1856a). Wood (1837) reported cholesterol in pus, and Lehmann (1850b) observed it in smegma. Vogel (1841, 1843, 1847) described deposits of cholesterol as occurring between the inner and middle coats of atheromatous arteries. Lindenmeyer (1863) was aware of its occurrence in cod liver oil, from which it was again prepared

[1] The so-called seroline was a mixture of cholesterol with fats, etc., according to Gobley (1851b, 1852).

in 1881 by Allen and Thomson. Its occurrence in human milk was demonstrated by Tolmatscheff (1867).

Additional references to the early investigations on cholesterol are given in the textbooks of Robin and Verdeil (1853), Gmelin (1871), and v. Fehling (1876). In the past half century studies have been so numerous, especially on distribution in health and disease and on the chemistry, that in a short review one can mention them only in instances of special significance. The reviews by Windaus (1908), Dubosc (1916), and Minovici (1927), and the special articles in Lewkowitsch (1921), Abderhalden (1925 *et seq.*), and Peters and Van Slyke (1931) cover much recent work on cholesterol and the vegetable sterols.

*Cholesterol in the lower animals.* Impressed by the constant occurrence of cholesterol in the organs and fluids of the higher animals, Dorée (1909) investigated its distribution lower down in the animal kingdom. He found it in the reptiles (snake), fishes (mackerel; *cf.* carp, cod, and salmon above), mollusks (whelk; *cf.* Henze, 1908a, on octopods, arthropods (crab), annulates (earthworm), and cœlenterates (sea anemone). He failed to detect it in the insects (*Bombyx mori*, the silkworm) and echinoderms (starfish), which, however, gave sterols resembling it.

In a contemporaneous study Welsch (1909) found cholesterol in two insects, *Cantharis vesicatoria* (Spanish fly) and *Melolontha*. He reported it also in the octopus (*cf.* Henze), and in a platyhelminth (*Bothriocephalus*, one of the tapeworms). Menozzi and Moreschi (1910) showed that even *Bombyx* contains cholesterol, in addition to its characteristic "bombicesterol." Mathews (1913) found much cholesterol in the bodies of starfish, and in the eggs of another echinoderm, the sea urchin.

In the porifera, or sponges, representing the borderline between metazoa and protozoa, Dorée found no cholesterol, but instead three new members of the "spongosterol" group of sterols first noted by Henze (1904, 1908b). Even in the protozoa cholesterol may be present. Panzer (1911) obtained masses of *Goussia gadi* from the air bladders of pollack, and from these coccidia he isolated a sterol apparently identical with cholesterol. It is not improbable, however, that the sterol came from the host, rather than from the parasite.

Bergmann (1934a, b), studying the sterols of mollusks, found in the large gastropods cholesterol admixed with a new, doubly unsaturated, sterol which he named ostreasterol. In some of the bivalves, particularly the oyster, *Ostrea virginica*, cholesterol appeared to be entirely replaced by ostreasterol.

From the foregoing evidence, it is apparent that cholesterol occurs

in the most diverse phyla of the animal kingdom, and if one can reason from the general to the specific, it would appear to be absent in no metazoa except the sponges and certain mollusks, and in these to be represented by one or another of the allied spongosterols or ostreasterols.

*Formula of cholesterol.* The currently accepted formula of cholesterol, $C_{27}H_{46}O$, was brought out by Reinitzer (1888) through the analysis of cholesteryl acetate dibromide. It has been confirmed by Obermüller (1890), by Windaus, v. Werder, and Gschaider (1932), and others. The existence of a double bond in the molecule is indicated by the formation of the dibromide (Wislicenus and Moldenhauer, 1868).

*Coprosterol.* The sterol in the feces of man was recognized as distinct from cholesterol as early as 1862 by Flint, who named it stercorine. Bondzyński (1896) rediscovered it and gave it the name koprosterin, or coprosterol, by which it is now designated. Bondzyński and Humnicki (1896) established for it the formula $C_{27}H_{48}O$ by analyzing several of its esters. Coprosterol is thus a dihydrocholesterol—one of several isomeric bodies possessing this formula. It is not, however, the normal dihydrocholesterol, for it is the reduction product of allocholesterol, a stereoisomer of cholesterol (Windaus, 1927).

Allocholesterol is not known to occur in nature, but since it is readily prepared by the action of hydrochloric acid on cholesterol, to which it also spontaneously reverts, its transient existence in body tissues, as the parent substance of coprosterol, may be considered probable. Windaus and Neukirchen (1919) had earlier observed that the hydrocarbon, coprostane or pseudo-cholestane, of which coprosterol is the alcohol, becomes cholanic acid when a carboxyl group suitably enters its aliphatic side chain. Cholanic acid in turn is the prototype of the bile acids.

*Dihydrocholesterol.* The normal dihydrocholesterol, or β-cholestanol, accompanies coprosterol in feces, but only to a small extent (Windaus and Uibrig, 1915). It also appears with coprosterol in the reduction of allocholesterol, again to a small extent (Windaus, 1927). In 1930 Schönheimer, v. Behring, Hummel, and Schindel discovered that dihydrocholesterol, but not coprosterol, is present as a contaminant in ordinary cholesterol. This fact is one of considerable physiological significance.

*Lanolin sterols.* At this point one may mention the so-called isocholesterol found by Schulze (1872, 1873a, b) admixed with cholesterol in wool grease, or lanolin. Isocholesterol has recently been shown by Windaus and Tschesche (1930) to be actually a mixture of agnosterol, $C_{30}H_{48}O$, and lanosterol, $C_{30}H_{50}O$, neither of which is isomeric with

cholesterol. The water-holding power of lanolin is sometimes cited as evidence of a rôle which sterols may play in the physical chemistry of protoplasm, but here analogy is perhaps more conspicuous than experimental evidence.

*Phytosterols—general.* Not only animal sources, but various vegetable materials have long been known to yield sterols. Braconnot (1811) isolated from mushrooms an "adipocire" which Vauquelin (1813) and Gobley (1856b) termed agaricine and Tanret (1889) named ergostérine (ergosterol). Beneke (1862a, b), Ritthausen (1863), Lindenmeyer (1863), Hoppe-Seyler (1866), and Ludwig (1869) obtained sterols from the unsaponifiable fractions of several vegetable fats, but they failed to recognize them as being other than cholesterol.

In 1878 Hesse prepared from Calabar beans and peas a sterol indistinguishable from the "cholesterol" which Beneke (1862a, b) had previously obtained from peas. Hesse observed that while it seemed to be isomeric with animal cholesterol it clearly was not identical with it. Cholesterol having been associated generally with animal sources, Hesse named the new vegetable substance phytosterol. Windaus and Hauth (1906) proved that the original Calabar bean phytosterol is actually a mixture of two other sterols, sitosterol and stigmasterol. The recent work of Sandqvist and Gorton (1930), Sandqvist and Bengtsson (1931), and Windaus, v. Werder, and Gschaider (1932) has indicated for sitosterol the formula $C_{29}H_{50}O$, and for stigmasterol the formula $C_{29}H_{48}O$. Thus neither substance is an isomer of cholesterol. This fact is important in considering the physiological relationships of the sterols.

*Sitosterol.* The old term, phytosterol, remains in current usage, but with a loose meaning which connotes a crude sterol of vegetable origin (Thoms, 1897), especially one that consists mainly of sitosterol. Sitosterol itself is the commonest vegetable sterol. Since its discovery by Burián (1897) in wheat and rye germ oils it has usually been regarded as an entity. Nevertheless, the work of Anderson and Shriner (1926), Anderson, Shriner, and Burr (1926), and Anderson, Nabenhauer, and Shriner (1927) indicates that it exists in at least three isomeric forms, and is usually accompanied by small amounts of dihydrositosterol, the physiological significance of which is unknown. The three isomeric sitosterols, $\alpha$, $\beta$ and $\gamma$, are so intimately associated, and so difficultly separated, that one is perhaps still justified in considering them as a single substance. Anderson and Moore (1923) have commented on the fact that although the phytosterols occur in all parts of plants, their relatively great abundance in seeds and pollen would seem to justify

the assumption that they play an important rôle in the life of the plant cells.

Several other sterols have been found in the higher plants. When Windaus and Hauth fractionated phytosterol it was felt that the number of individual sterols would diminish as mixtures were resolved into their components. The actual tendency is otherwise; sterol molecules are large, and new isomers and homologs continue to be discovered. Some of these cannot yet be discussed physiologically, and are therefore omitted from this review.

The major unsaponifiable substance of grass was unhappily designated chortosterol by Dorée and Gardner (1908). It was shown to be identical with the so-called hippocoprosterol isolated from horse dung by Bondzyński and Humnicki (1896). Pollard, Chibnall, and Piper (1931) and Bills and Steel (1933) identified it as ceryl alcohol, the same as found in spinach leaves by Collison and MacLean (1931), and probably a significant item in the diet and feces of most herbivora. Since ceryl alcohol is no sterol, being merely a mixture of higher fatty alcohols, investigations in which it appears to have been confused with the true fecal sterols are here omitted.

*Discovery of ergosterol.* In 1879 Tanret noticed that the crude sterol obtained from a fungus, ergot, was more strongly levorotatory than cholesterol. Pursuing this observation, he found (1889, 1890) that the characteristic ergot sterol, which he named ergostérine (ergosterol), differs also in composition from cholesterol and the phytosterols. Still later (1908a, b) he succeeded in preparing ergosterol almost free from contaminant fungus sterols which are separable only with difficulty. With this material he gave a description of the constants of ergosterol which was so accurate that with slight changes it holds good today. His formula for ergosterol, $C_{27}H_{42}O$, is frequently cited, but the formula $C_{28}H_{44}O$, recently given by Windaus and Lüttringhaus (1932), is more probably correct.

*Ergosterol in the lower plants.* Following the work of Hesse and of Tanret, Gérard (1892, 1895a, b, 1898) studied the sterols from a number of higher and lower plants. He was struck by a taxonomic relationship in the occurrence of sterols, which led him to propound the rule that phytosterol is characteristic of the higher plants, or phanerogams, whereas ergosterol is characteristic of the lower plants, or cryptogams, just as cholesterol is the typical sterol of animals. Gérard's rule holds good in a general way, but there are several exceptions to it, and its physiological significance is obscure. His experiments greatly extended

knowledge of the distribution of sterols, especially of ergosterol in the lower plants, for they included a representative each of the brown algae, slime fungi, bacteria (staphylococci), mucors, yeasts, penicillia and lichens. In some instances his identification of the sterol as ergosterol was admittedly incomplete, yet recent determinations have shown the decisions to be substantially correct.

It is historically interesting that the mushroom substance of Braconnot, Vauquelin, and Gobley almost certainly was ergosterol. Also it seems that ergosterol had been dealt with by Ludwig (1869), who worked with ergot, and by Hoppe-Seyler (1871) and v. Nägeli and Loew (1878), whose starting material was yeast. These workers had confused it with cholesterol; Ludwig because of reliance on a color reaction, and Hoppe-Seyler because of its superficial resemblance to some cholesterol he had obtained from pus cells. The description of the crude yeast sterol by v. Nägeli and Loew as silky needles smelling of geranium and beeswax is exactly that of ergosterol contaminated with an odorous yeast derivative which even in technical practice is difficult to remove.

Gérard's recognition of ergosterol in yeast and molds has been substantiated by many later workers, and these two sources today supply most of the ergosterol of commerce. Additional examples of the occurrence of ergosterol in mushrooms are given by Zellner (1907) in his book on the chemistry of the higher fungi, and by several later investigators.

In the cryptogams, or lower half of the plant kingdom according to the botanical classification used by Gérard, the club moss, *Lycopodium*, represents one of the higher orders. Bukovski (1889) investigated the oil from *Lycopodium* spores, finding therein a sterol resembling phytosterol. From his description, one can be fairly certain that this sterol did not consist predominantly of ergosterol. Gérard's rule would therefore be better stated if it limited the predominance of ergosterol to the lower orders of cryptogams.

*Sterols of bacteria.* Certain bacteria are perhaps the only organisms without a sterol of any sort. Anderson and Chargaff (1929) obtained negative color reactions for sterols in the unsaponifiable matter of *Mycobacterium tuberculosis*, thus confirming Kresling (1901), Panzer (1912), and Goris (1920) who found not more than a doubtful trace. Prickett and Massengale (1931) got negative findings in the sensitive spectrographic test for ergosterol in six species of *Mycobacterium*. v. Behring (1930) found no sterol in *Bacterium coli* and the diphtheria bacillus. On the other hand *Bacillus butyricus*, investigated by Heiduschka and Lindner (1929), yielded ergosterol, as did *Staphylococcus*

*albus* (Gérard, 1898). It is strange that the mycobacteria, which somewhat resemble the molds, should lack ergosterol, while at least two simpler forms contain it.

*Minor fungus sterols.* Tanret (1908a, b) recognized that ergosterol is not the only sterol in fungi. It is one of the so-called mycosterol group of sterols, which have been studied most thoroughly in ergot and yeast. From ergot oil Heyl and Swoap (1930) isolated $\alpha$-dihydroergosterol similar to that obtained by Windaus and Brunken (1928) in the reduction of ergosterol itself. Heyl (1932) later found an unnamed third sterol which is isomeric with $\alpha$-dihydroergosterol, and which when admixed with the latter apparently constitutes what Tanret termed "fongistérine." Yeast contains a number of minor sterols. Of these, $\alpha$-dihydroergosterol (Callow, 1931b) and zymosterol (MacLean, 1928) are the most abundant; cerevisterol (Honeywell and Bills, 1932, 1933) is present in very minute amount. Several other new sterols were reported in yeast by Wieland and Asano (1929) and Wieland and Gough (1930), but at least one of these appears to be a mixture.

*Ubiquitous occurrence of "ergosterol."* With a single exception, no vegetable sterol has been found in animal cells, and no animal sterol in plant cells. The exception is ergosterol, or a sterol very similar to it, which *in traces* occurs admixed with both cholesterol and phytosterol of the most diverse origins. This singular universality in distribution was revealed spectrographically in researches leading to and following the discovery of the convertibility of ergosterol into vitamin D by irradiation. Data from several spectrographic determinations are given in table 1, from which it is evident that the ergosterol admixture in cholesterol usually runs less than 5 parts per 1000. Somewhat larger amounts may occur in the phytosterols of the higher plants, yet these quantities are small in comparison with the concentrations attained in the fungi. It may be noted finally that the almost universal occurrence of "ergosterol" is indicated by the very wide variety of edible substances which acquire the antiricketic property of vitamin D upon irradiation.

*Vitamin D.* A century of experience with cod liver oil as a remedy for rickets was reviewed by Hess (1929). The prophylactic value of this oil was demonstrated by Hess and Unger (1917). Mellanby, in a series of experiments which were summarized in 1921, indicated that rickets, in dogs, is a dietary deficiency disease, and that cod liver oil contains an antiricketic vitamin. Proof of the calcifying action of cod liver oil in rat rickets was contributed by Shipley, Park, McCollum, Simmonds,

TABLE 1

*Spectrographic determinations of the occurrence of "ergosterol" in traces admixed with the total sterol of various animal and higher plant sources*

| ORIGIN OF STEROL | RATIO OF "ERGOSTEROL" TO TOTAL STEROL | REFERENCE |
|---|---|---|
| | *parts per 1000* | |
| Unspecified (cholesterol).... | 0.2 | Pohl (1927) |
| Skin, pig..... | Present | Rosenheim and Webster (1927b) |
| Brain..... | 0.5 | Rosenheim and Webster (1927c) |
| Gallstones..... | Present | Rosenheim and Webster (1927c) |
| Cottonseed oil..... | 7.5 to 25.0 | Heilbron, Kamm and Morton (1927) |
| Maize oil..... | Present | Heilbron, Kamm and Morton (1927) |
| Peanut oil..... | Present | Heilbron, Kamm and Morton (1927) |
| Linseed oil..... | Present | Heilbron, Kamm and Morton (1927) |
| Spinal cord, cattle..... | 1.2 | Bills, Honeywell and MacNair (1928) |
| Gallstones, man..... | Present | Bills, Honeywell and MacNair (1928) |
| Skin, man..... | Present | Heilbron (1928) |
| Blood, man..... | Present | Dejust, Van Stolk and Dureuil (1928) |
| Cod liver oil..... | Present | Woodrow (1928) |
| Butter..... | Present | Adam (1928) |
| Preen gland of birds..... | Present | Hou (1928) |
| Brain, Coptic mummy..... | Present | King, Rosenheim and Webster (1929) |
| Plankton, marine..... | Present | Belloc, Fabre and Simonnet (1930) |
| Milk, cow..... | 2.3 | Hentschel and Bachmann (1930) |
| Skin, infant's..... | 1.5 | Hentschel and Schindel (1930) |
| Skin, adult's..... | 4.2 | Hentschel and Schindel (1930) |
| Venom of toads..... | 2.0 | Chen, Jensen and Chen (1931) |
| Venom of toads..... | 3.0 to 4.7 | Chen, Jensen and Chen (1933a, b) |
| Venom of toads..... | 0.0 to 13.6 | Chen and Chen (1933a, b, c) |
| Brain, human fetus..... | 0.5 (av.) | Page and Menschick (1931) |
| Brain, human infant..... | 0.2 (av.) | Page and Menschick (1931) |
| Brain, human adult..... | 0.06 (av.) | Page and Menschick (1931) |
| Mice, gutted carcasses..... | 0.8 | Schönheimer, v. Behring and v. Gottberg (1932) |
| Rats, gutted carcasses..... | 0.3 | Schönheimer, v. Behring and v. Gottberg (1932) |
| Brain, rabbit..... | 0.7 | Schönheimer, v. Behring and v. Gottberg (1932) |
| Blood, dog..... | 0.1 | Schönheimer, v. Behring and v. Gottberg (1932) |
| Lymph, dog..... | 0.3 | Schönheimer, v. Behring and v. Gottberg (1932) |
| Sclerotic aortas, man..... | Absent | Schönheimer (1932) |
| Egg yolk..... | 1.0 | Schönheimer and Dam (1932) |

and Parsons (1921), and in human rickets by Park and Howland (1921).

Zucker, Pappenheimer, and Barnett (1922) found that the antiricketic vitamin resists alkali, appearing in the unsaponifiable, *i.e.*, sterol fraction of the oil. They suggested "that it may be a sterol related to cholesterol or a cholesterol derivative." Many subsequent attempts to isolate the vitamin from cod liver oil by saponification have led to nothing better than concentrates a few hundred times more potent than the starting material. The characterization of this vitamin as a sterol received support in the work of Hess, Weinstock, and Helman (1925) and Steenbock and Black (1925) which showed that sterols develop antiricketic potency upon exposure to ultraviolet rays. Nevertheless, the identity of the vitamin from fish oil with that of irradiated sterols has never been established, and indeed there is now much evidence that the two forms are dissimilar.

Early investigations on the antiricketic vitamin suffered from the multiplicity of factors which are concerned with rickets—species of animal, rate of growth, quantity and ratio of minerals, intensity and wavelength of light, availability of vitamin, etc. The existence of the vitamin as a substance distinct from vitamin A was demonstrated by McCollum, Simmonds, Becker, and Shipley (1922) in one of a series of experiments which cleared up much of the antecedent confusion. These workers by aërating hot cod liver oil destroyed the antixerophthalmic factor, vitamin A, leaving intact an antiricketic factor which they later (1925) termed vitamin D. Collateral experiments of the Baltimore investigators and others are discussed in the review on the etiology of rickets by Park (1923) and in the textbook by McCollum and Simmonds (1929). The chemistry of cod liver oil has been reviewed by Bills (1927a).

The development of assay technique employing the rat as test animal has been followed by many investigations on the occurrence of vitamin D. A rickety rat will respond to less than 1 part per billion of vitamin D in its diet; still smaller amounts can be detected in edible materials by assaying the unsaponifiable fractions. For comparison, it may be mentioned that the animal test for vitamin D is approximately 1000 times more sensitive than the delicate spectrographic test for ergosterol. It is all the more interesting, therefore, to find that the distribution of vitamin D is far less in both amount and extent than that of ergosterol.

Mellanby (1921) and particularly Hess (1923) found that egg yolk contains a significant amount of vitamin D. McCollum, Simmonds,

Becker, and Shipley (1922) found vitamin D not only in cod liver oil, but in the liver oils of the burbot and shark. Bills (1924) determined that the body oils of fish, as well as their liver oils, contain vitamin D. He found menhaden body oil comparable in potency to cod liver oil. Hess and Weinstock (1926) studied samples of puffer fish liver oil that were more potent than cod liver oil. Bills (1927b) made the first quantitative comparisons on an extensive range of oils. Among the 27 kinds submitted to assay were 14 fish liver or body oils, all potent though in varying degree. The body oil of a cephalopod mollusk, squid, exhibited slight, yet definite potency. Bills observed that whale blubber oil was inactive, and that the blubber oil of another marine mammal, the seal, was only faintly active. Veal fat and the oleo oil of cattle were inactive. The common vegetable oils gave no evidence of vitamin D.

Nearly all fish oils contain vitamin D. Table 2 is a revised compilation of assays made in the author's laboratory on the oils from many species of fish. The list could be extended considerably by including scattered assays from other sources, but the present collection is sufficiently illustrative, and has the advantage that the data are comparable because obtained under a uniform technique. Since vitamin D has never been isolated from fish oils, and there is evidence that the crystalline active substance obtained from irradiated ergosterol is only one of several forms of the vitamin, it is customary to report assays not in gravimetric terms, but in units. The system of units adopted by the leading workers is the international, or League of Nations, system. Since it happens that the potency of medicinal cod liver oil is approximately 100 international units per gram, one can compare at a glance the potencies of the other oils given in table 2.

In spite of ample demonstration to the contrary, the popular impression persists that milk, butter, and leafy vegetables are practical sources of vitamin D. This notion is doubtless a holdover from the early confusion of vitamin D with vitamin A, or a result of faulty technique which was common in early experiments. Even today occasional workers in experimental rickets sometimes overlook the fact that not much more than 10 per cent of test substance can be added to the standard rickets-producing rations without danger of producing a false test, *i.e.*, calcification not due to the vitamin. Such additions may affect the absolute or relative Ca:P content of the diet, and otherwise alter acid-base relations, or they may affect the bacterial flora of the rat's intestine, altering the pH, or, particularly in the case of oils and fats,

TABLE 2

*Distribution of vitamin D in various fish oils*

| SOURCE OF OIL | ZOOLOGICAL NAME | POTENCY, I.U. PER GM. |
|---|---|---|
| Bluefin tuna, liver | *Thunnus thynnus* | 40,000 |
| Swordfish, liver | *Xiphias gladius* | 10,000 |
| Yellowfin tuna, liver | *Neothunnus macropterus* | 10,000 |
| Black sea-bass, liver | *Stereolepis gigas* | 5,000 |
| Bocaccio, liver | *Sebastodes paucispinis* | 2,100 |
| Red rockfish, liver | *Sebastodes ruberrimus* | 1,500 |
| Black rockfish, liver | *Sebastodes mystinus* | 1,500 |
| China rockfish, liver | *Sebastodes nebulosus* | 1,400 |
| "Ling cod" (not codfish), liver | *Ophiodon elongatus* | 1,300 |
| Chinook salmon, liver | *Oncorhynchus tschawytscha* | 1,300 |
| Halibut, liver | *Hippoglossus hippoglossus* | 1,200 |
| Rabbitfish, liver | *Chilomycterus schœpfi* (?) | 1,100 |
| Striped rockfish, liver | *Sebastodes elongatus* | 1,000 |
| Starry flounder, liver | *Platichthys stellatus* | 1,000 |
| Boston mackerel, liver | *Scomber scombrus* | 750 |
| "Black cod" (not codfish), liver | *Anoplopoma fimbria* | 600 |
| Pufferfish, liver | *Sphœroides maculatus* | 570 |
| Dog salmon, liver | *Oncorhynchus keta* | 400 |
| Black horse, mesentery | *Cycleptus elongatus* | 400 |
| Turbot, liver | *Reinhardtius hippoglossoides* | 260 |
| Rex sole, liver | *Errex zachirus* | 150 |
| California sand dab, liver | *Orthopsetta sordida* | 120 |
| Cod, liver | *Gadus morrhua* | 100 |
| Herring, entire body | *Clupea harengus* | 100 |
| Yellow sole, liver | *Pseudopleuronectes dignabilis* | 90 |
| Sardine, entire body | *Sardinia cærulea* | 80 |
| Goosefish, liver | *Lophius piscatorius* | 70 |
| Pollack, liver | *Pollachius virens* | 50 |
| Menhaden, entire body | *Brevoortia tyrannus* | 50 |
| Shark, liver | (Sp.) | 50 |
| Salmon, trimmings | *Oncorhynchus* (Sp.) | 40 |
| Turbot, body minus liver | *Reinhardtius hippoglossoides* | 30 |
| Skate, liver | *Raja binoculata* | 25 |
| Dogfish (Pacific), liver | *Squalus suckleyi* | 20 |
| Muddy catfish, body | *Leptops olivaris* | 20 |
| Ohio perch, mesentery | *Aplodinotus grunniens* | 11 |
| Buffalo, mesentery | *Ictiobus cyprinella* | 10 |
| Haddock, liver | *Melanogrammus æglefinus* | 10 |
| Channel catfish, mesentery | *Ictalurus punctatus* | 5 |
| Dogfish (Atlantic), liver | *Squalus acanthias* | 3 |
| Capelin, entire body | *Mallotus villosus* | 3 |
| Ratfish, liver | *Chimæra colliei* | 2 |
| Gray sole, liver | *Glyptocephalus cynoglossus* | <1 |
| Sturgeon, liver | *Acipenser rubicundus* | nil |

they may abstract calcium from the gut in the form of soaps. Assays of butter are particularly subject to error (Kon and Booth, 1934).

When one disregards experiments faulty in these ways one finds that the vitamin D potency of butterfat is normally very low. It may be increased considerably by giving the cow a fish oil or irradiated yeast. By the same standards, there seems to be no sound evidence that vitamin D ever occurs in live plant tissue. Dead tissue, by insolation, may acquire slight potency, as in the case of hay cured in the sun, coconut oil rendered by solar rays, or mushrooms struck by sunlight. In the case of a dried yeast rich in ergosterol, Massengale, Bills, and Prickett (1931) found that brief exposure to the sun developed a potency 100 times that of cod liver oil. In theory at least, the somewhat widespread occurrence of yeasts, as on the skin of fruit, might result in the formation of traces of vitamin D, but here again the vitamin would presumably be formed in dead plant substance, accidentally as it were, because yeasts, like mushrooms, are destroyed by direct sunlight.

There is no evidence that vitamin D plays any rôle in plant physiology, and it is not difficult to understand why it is not found in live plant tissues. The lower, non-pigmented, non-photosynthesizing plants, which contain much ergosterol, thrive in dark places and perish in the light. The higher plants, containing relatively little ergosterol, possess pigments which presumably filter out the activating rays at the short end of the solar spectrum. Even if the vitamin could be formed in the superficial layers, plants could not store it, "unprovided as they are with any system for the translocation of lipoids."

GENERAL CHEMICAL CONSIDERATIONS. In the preceding section the distribution of sterols is reviewed with incidental mention of certain chemical relationships. Before the physiological topics are taken up it is well to consider briefly and generally some fundamental chemical qualities of the sterol family. Sterol chemistry has been investigated in greater detail than sterol physiology, but the intention here is to recall merely the salient facts, and particularly certain developments of the past two years.

*Esters, glucosides, and complexes.* In nature the sterols are associated with the fats, to which they bear some resemblance in physical properties, but from which they are very different chemically. As alcohols, they form esters with the fatty acids *in vitro* and *in vivo*. Berthelot (1859) synthesized several cholesterol esters, and predicted their discovery in the body. Schulze (1872, 1873a, b) discovered cholesterol esters in wool grease, and Hürthle (1896) proved the existence of several

cholesterol esters in the blood. Page and Rudy (1930) have summarized and corrected the extensive chemical literature on the fatty acid esters of cholesterol. Natural glucosides of the phytosterols, the phytosterolins, were described by Power and Salway (1913). Artificial *d*-glucosides of sitosterol and cholesterol were prepared by Salway (1913). Ergosterol *d*-glucoside was made by MacCorquodale, Steenbock, and Adkins (1930). A conjugated cholesterol was reported in urine by Gardner and Gainsborough (1925).

A significant property of the sterols is that besides entering into ordinary chemical unions of the types just described, they exhibit a tendency to form loose associations with each other, their own esters and derivatives, the bile acids, the saponins, and with such unrelated substances as urea, substituted ureas, glycerol, maleic and citraconic anhydrides, water and the organic solvents of crystallization. Upon this property hangs the difficulty of obtaining them pure, and upon it presumably depends some of their importance physiologically.

The formation of complexes with bile acids, *i.e.*, the "choleic acid reaction," has been associated with the fact that some bile acids, such as desoxycholic acid, appear to promote the absorption of sterols. In the experiments of Schönheimer (1924), Loeffler (1928), and Hummel (1929) the absorption, or at least the retention, of cholesterol was markedly increased by the simultaneous administration of a bile acid. Hummel questioned the assumption that the effect is actually due to the formation of complexes, for he observed it with certain acids, such as cholic acid, which do not form choleic acids. The action, he suggests, may be merely a hydrotropic effect.

The formation of complexes with saponins is the basis of the most important analytical reaction of the sterols—the precipitation of insoluble digitonides (Windaus, 1909, 1910), by which certain sterols can be separated from others, from their esters, and from foreign matter.

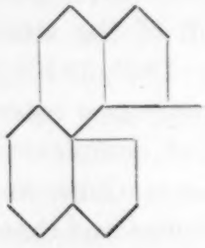
Evidence of the existence of complexes of cholesterol with proteins has appeared in several investigations, and as regards the proteins of blood this work has been reviewed and extended by Gardner and Gainsborough (1927b). MacCorquodale, Steenbock, and Adkins (1930) observed that defatted yeast is antiricketically activatable, a fact which indicates that ergosterol may exist in combination with protein. Schoenheimer and Breusch (1933) noted that some of the sterol of wool is so intimately bound to the protein that ether does not extract it.

*Solubilities.* Sterols are soluble in most organic solvents, but they are insoluble in water. They can be put into colloidal aqueous solution,

electronegative, by means of a mutual vehicle such as acetone, or by treatment in a colloid mill, or by the action of supersonic waves (unpublished experiment). They dissolve to a small extent in bile or soap solution. Their acid esters with dibasic acids, *e.g.*, phthalic acid, form sodium or potassium salts which readily dissolve in water giving clear, frothy solutions.

*Biochemical relationships.* Although many sterols have received intensive chemical study, physiological knowledge of these bodies is limited to the commonest few, particularly to cholesterol and the isomer of ergosterol called vitamin D. The chemical investigations have revealed that the sterols are related to other physiologically important substances (Pryde, 1934), notably the bile acids (Windaus and Neukirchen, 1919; Heilbron, Simpson, and Spring, 1933), the follicular and pregnancy hormones and perhaps a testicular hormone (Butenandt, 1931, 1932; Butenandt, Weidlich, and Thompson, 1933; Bernal, 1933b; Cook, Dodds, and Hewett, 1933; Danielli, Marrian, and Haslewood, 1933). They are probably related to the bufotoxin of toad venom (Wieland and Alles, 1922; Wieland, Hesse, and Meyer, 1932; Chen and Chen, 1933d), to the digitalis and strophanthus alcohols (Jacobs and Fleck, 1932; Jacobs and Elderfield, 1932; Jacobs, 1933; Rosenheim and King, 1933; Bernal, 1933a), and to the carcinogenic hydrocarbons (Cook, 1932; Rosenheim and King, 1933).

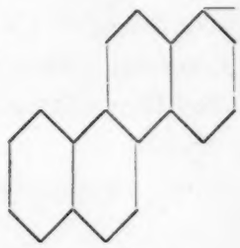
*Ring structure.* The hydrocarbon skeleton common to the sterols is one of the most massive ring systems with which living cells have to deal. The classic terpenoid representation of this ring system (I) has in recent years met with theoretical objections, culminating in the x-ray



I

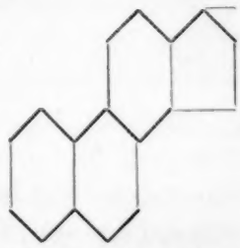
crystal measurements of Bernal (1932). Bernal showed the old formula to be untenable; the structure must be more elongated.

The newer knowledge led Rosenheim and King (1932) to the revolutionary view that the sterol ring is chrysenoid (II).



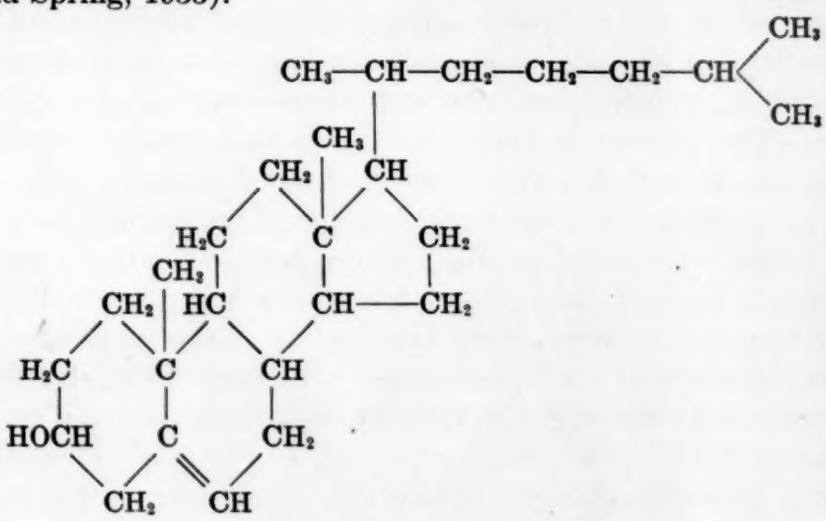
II

Formula II soon came under question as a result of more critical fact-fitting by Wieland and Dane (1932), Windaus (1932a), Butenandt (1932), and others. Heilbron, Simpson, and Spring (1933) have reviewed the chemical argument which, in a word, leads to the following phenanthrene-cyclopentane, or "cholane," structure (III) as the most probable form of the sterol ring.



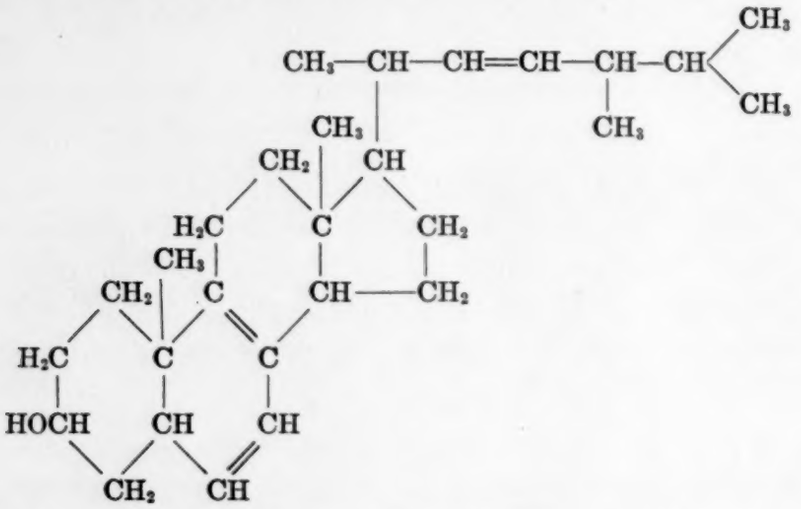
III. Sterol skeleton

The details of location of substituent groups and especially of the double bonds, upon which the physiological specificities of the sterols depend, are still in process of being thrashed out. Subject to change, the latest formula for cholesterol is IV (Windaus, 1933; Heilbron, Simpson, and Spring, 1933).




IV. Cholesterol (tentative)

A recent formula for ergosterol is V (Windaus and Langer, 1933). Variations are proposed by Chuang (1933), Windaus (1933), Heilbron, Simpson, and Spring (1933), and Rosenheim and King (1934).



V. Ergosterol (tentative)

Synthesis of the sterol ring-system has been accomplished by Cook and Hewett (1933a, b), Ruzicka, Ehmann, Goldberg, and Hösli (1933), and Kon (1933).

For structural formulas and relations of other sterols and bile acids, interpreted on the basis of the new skeleton, the reader is referred to the excellent paper by Windaus (1932b). For accounts of the development, year by year, of the intricate chemical knowledge of the sterols, one of the best sources is the *Annual Reports* of the Chemical Society, London.

Differences in the chemical and physiological properties of sterols are associated with side chain substitutions and rearrangements at double bonds, rather than with any deep-seated alterations in the nucleus. The nucleus indeed is so stable that drastic treatment is required to rupture it. The sterol molecules contain, besides alkyl groups attached to the rings, 1 or 2 hydroxyl groups and from 0 to 3 double bonds. Hydroxyl groups and the double bonds are centers of isomerization and chemical change, and so one finds the sterols in spite of their ring system very active chemically. Their physiological importance, however, is often determined with great specificity by some slight structural difference, *e.g.*, the stereoisomerism between ergosterol and vitamin D.

ORIGIN, ABSORPTION, AND EXCRETION OF STEROLS. *In the lower plants.* An indication that the formation of sterol in the lower plants

can occur at any stage of the life cycle is given in the work of Sumi (1933). He observed that the ergosterol percentage of a mushroom, *Cortinellus shiitake*, steadily rose from the button stage to the over-ripe stage.

It is easily demonstrated that the lower plants synthesize sterols from simple compounds of carbon. Gérard (1892) obtained ergosterol from *Penicillium glaucum* cultivated on the medium of Raulin (1869) in which sucrose and tartaric acid are the carbon sources. A sterol, probably ergosterol, was extracted by Gérard (1895a, b) from *Mucor mucedo* grown on a medium in which lactose supplied the carbon. Pruess, Peterson, Steenbock, and Fred (1931) extracted the sterol from a number of fungi cultivated on a medium in which the carbon source was glucose. Birkinshaw, Callow, and Fischmann (1931), working with *Penicillium puberulum*, and Pruess, Peterson, and Fred (1932), with *Aspergillus fischeri*, identified as ergosterol the sterol produced from glucose as the carbon source.

It has long been known that different fungi yield different percentages of ergosterol. Experiments prove that the variation is related, as one would expect, to both species and conditions of culture. Bills, Massengale, and Prickett (1930) cultivated 29 yeasts in a given medium, under uniform conditions, and observed that these yeasts exhibited decidedly different capacities for the elaboration of ergosterol. The extremes were shown by *Saccharomyces logos*, which gave only a trace, and *Saccharomyces carlsbergensis*, which yielded 2 per cent of ergosterol.

In a second experiment Massengale, Bills and Prickett (1931) demonstrated that a given yeast, *Saccharomyces cerevisiæ*, elaborated widely different amounts of ergosterol, according to the sugar which supplied carbon to the basal medium. This work contributes one of the few bits of information available on the mechanism of sterol formation. Seven fermentable sugars were used, with the finding that di- and trihexoses occasioned the synthesis of much greater amounts of ergosterol than did the monohexoses (singly or in admixture) into which they are hydrolyzed. The amounts of ergosterol produced bore no relation to the protein or fat content of the yeast cells. It is a reasonable inference, therefore, that the monohexoses which are split from the higher sugars by the yeast enzymes exist at the instant of cleavage in forms particularly suitable for synthesis into sterol. According to this concept, sterols are primarily products of carbohydrate metabolism.

In this connection, an experiment by MacLean and Hoffert (1926) is significant. They found that the presence of sulphite in the sugar

solution in which yeast was incubated resulted in a marked decrease in sterol production but in little or no interference with the elaboration of fatty acid. The argument was advanced that in the formation of sterol, but not of fatty acid, an aldehyde removable by sulphite comes into the picture between the hexose and the final product.

Terroine, Bonnet, Kopp, and Vechot (1927) performed experiments with the mold, *Sterigmatocystis nigra*, which is capable of utilizing either peptone, sugar, or fat as the sole source of carbon. With various fats as the source, sterol was elaborated, actually in greater quantity, than with peptone or glucose as the source, but there was no parallelism between the amounts of sterol and fatty acid synthesized in the several cultures. These facts were taken to indicate that the sterols are formed at the expense of fats, rather than as an accompaniment to them. Certainly it is obvious that the carbon of the fats became the carbon of the sterols in this instance, but the same argument can be applied to the glucose or the peptone, as the case may be. The greater quantity of sterol in the mold grown on fat has little significance, because cultural conditions other than the source of carbon presumably must have been varied in the cultivation of the mold on peptone or glucose. It is possible that the formation of sterol from fat involved the intermediation of a carbohydrate or aldehyde.

*In the higher plants.* The formation of sterols in the higher plants, as in the lower, can take place at any stage of development. The storage in mature plants is well illustrated by the experiment of Lindenmeyer (1863), which demonstrated that the seeds of the pea accumulate sterol as they ripen. The ability to synthesize sterols appears early in young plants. Beneke (1866), Schulze and Barbieri (1882), Schulze (1890), and Terroine, Bonnet, Kopp, and Vechot (1927) demonstrated that the sterol percentage of seeds usually increases during sprouting, even in the dark. The latter investigators assumed that the sterol originates at the expense of fat stored in the seed—a view which would be convincing if accompanied by a demonstration that the sterol could not have come from some other constituent of the seed, or that the fat of the seed could not have gone for other conversions.

Very little is known regarding the origin of sterols in the higher plants. Minovici (1927) made the picturesque suggestion that these sterols are derived from agrosterol, a soil constituent which Schreiner and Shorey (1909) regarded as a product of decaying vegetation or mold action. Against this idea is the difficulty of absorption and transportation of a water-insoluble substance through plant tissues. Since plants

have no known mechanism for the translocation of lipoids, it would seem that their sterols must originate at the site of their accumulation.

It is more probable that the dihydrositosterol of plants originates by the reduction of sitosterol than that the reverse relation exists. In animals the corresponding reduction products of cholesterol predominate in fecal, *i.e.*, excretory, matter, and the direction of the relationship is obvious. In plants, which have no excretory mechanism for sterols, one finds a relatively greater concentration of reduced sterol. Certain resins, which might be regarded as plant excretions, contain saturated sterol-like substances. These facts, together with the general principle that reduction is the fundamental process in plant physiology, indicate the probability that, molecular structure permitting, the more reduced sterols come from the more unsaturated.

Heilbron and Sexton (1929) suggested that the ever-present ergosterol, which is trebly unsaturated, arises by an oxidation of sitosterol concurrent with a reduction to dihydrositosterol. Against this view is the preceding argument, and also a new difficulty: sitosterol is now considered to contain 29 carbon atoms, whereas ergosterol has only 28 carbon atoms.

*In animals.* The problem of the origin of sterols in animals is complicated by the fact that the food of animals is ultimately always of vegetable origin. The early investigators knew nothing of plant sterols, and they knew cholesterol chiefly as a component of pathological concretions and old necrotic tissues. They naturally regarded it as a product of morbid animal origin. Even Denis, who detected cholesterol in the blood in 1830, regarded its presence there as pathological.

This concept began to change with the discovery by Couerbe in 1834 that cholesterol is a major element of the brain. To him it appeared to be elaborated by the nervous system—a view which to this day has not been disproved, and is the hypothesis held by a few investigators.

Lehmann (1850a) regarded cholesterol as the decomposition product of some unknown precursor. He reasoned that the precursor was probably no fat, because in animal metabolism fats are usually oxidized, whereas to produce a substance having the formula of cholesterol the opposite process would be required. This is significant in view of the occasionally cited, but incomprehensible report of Lifschütz (1908), who thought he had produced a sterol by the oxidation of oleic acid.

Flint (1862) regarded cholesterol as an excrementitious product originating in the brain. He performed extensive experiments which, according to his lights, proved this origin. It was at this time that

Flint discovered coprosterol (stercorine). He found coprosterol in the normal feces of man and dog, but from the feces of a fasting dog he got only cholesterol, just as is found in the meconium and in the feces of hibernating animals. He confirmed Becquerel and Rodier (1844b) in the observation that in severe jaundice with liver involvement there is a rise in blood cholesterol. He concluded that coprosterol comes from cholesterol as a result of digestive action. He coined the term, cholesteremia (hypercholesterolemia), and observed that this may occur, even without jaundice, in liver disease.

Compared with present methods, Flint's analytical procedures were imperfect, and he sometimes drew conclusions from comparatively slight differences. Some of the conclusions given in his historically important paper, but later disproven, are omitted here. But textbooks for many years reflected his somewhat too simple picture of cholesterol metabolism, namely, that cholesterol is produced in the brain and nervous tissue, carried by the blood, separated by the liver, voided by the bile, and converted into coprosterol in its passage down the gut.

Beneke (1880) indicated that Flint's view of the excrementitious significance of cholesterol is untenable. He found that the cholesterol content of the brain was 2.2 per cent on the wet basis, a quantity greater than one might reasonably expect of an excrementitious product. He found also that cholesterol occurs in the lymph vessels of the mesentery, in amounts far greater than in the food, the inference being that some of the supposedly excrementitious substance is resorbed rather than excreted.

In the immense literature since Beneke, the question of the site and mechanism of cholesterol formation has repeatedly come up, but no satisfactory answer has been given. Besides the brain and nervous system, several other organs have been suggested as the site of synthesis. It is unnecessary to repeat the scant evidence, already reviewed by Dorée and Gardner (1908), which pointed to the liver, the bile passages, or the red corpuscles. More recently the ovaries, the spleen, and particularly the adrenals have been considered by several French investigators. Their work has been reviewed and discredited by Baumann and Holly (1923), Joelson and Shorr (1924), and Randles and Knudson (1928), yet workers as recent as Chamberlain (1929, 1930) occasionally attach special significance to the adrenals.

When all the facts are considered, it appears as likely as not that animal sterols, in so far as they are endogenous, originate in the cells in which they occur. It has already been pointed out that this prob-

ably must be the case in plants. The fact that animals possess means for the transport of sterols is hardly evidence that animal cells individually lack the power to synthesize them.

As to the carbon source from which animal sterols arise, the available evidence is nil. Negatively, it can be pointed out that the association of sugar and sterol in diabetes (Rabinowitch, 1929; Hunt, 1929; and others) is no clue, for in this disease the blood picture for sterol, sugar, and fat moves more or less as a unit, and all three are influenced by insulin. Among the 200 or more references to cholesterol metabolism in the excellent review by Peters and Van Slyke (1931) are numerous citations which relate variations in blood cholesterol to various factors, clinically important, but not informative on the question of origin.

One school of thought, exemplified by Gardner, Minovici, Thannhauser, and their colleagues, formerly held that the sterol of animals is mainly exogenous in origin. This hypothesis was based on *a*, the extremely wide distribution of sterols in plants; *b*, the fact that animals are generally incapable of synthesizing heterocyclic compounds; and *c*, the belief (erroneous) that cholesterol and the phytosterols were isomeric. The egg-hatching experiments of Parke (1867), Beneke (1880), Mendel and Leavenworth (1908), Ellis and Gardner (1909), Mueller (1915a), Thannhauser and Schaber (1923), and Beumer (1925) showed, in general, that the cholesterol content of the newly hatched chick was not greater than that of the egg, thus indicating no synthesis. Moreover, the *absorption* of cholesterol, albeit not of phytosterol, was amply demonstrated. Thus Anitschkow and Chalatow (1913), Wacker and Hueck (1913), Rubinstein (1917), Anitschkow (1924), and Scarff (1927) showed that cholesterol fed to a susceptible animal, such as the rabbit, induces atheroma or atherosclerosis not unlike that described by Vogel (1841) in man. Additional demonstrations of cholesterol absorption are given by Beneke (1880), Jankau (1891), Pribram (1906), Kusumoto (1908), Gardner and Lander (1913), Wacker and Hueck (1913), Mueller (1915b, 1916), McMaster (1924), Arndt (1926), and others.

The current view is that cholesterol originates in the animal kingdom, and may pass from one animal to another. In other words, it is endogenous in herbivora after weaning, and either endogenous or exogenous in carnivora.

Synthesis in animals has been demonstrated in the feeding experiments with sterol-deficient diets conducted by Channon (1925), Randles and Knudson (1925), and others. This work substantiates the finding of a greater output than intake of sterol in the balance experiments of

Gamble and Blackfan (1920) with infants on a milk diet, and of Gardner and Fox (1921) with adults on mixed diets. Schönheimer (1929) showed that the laying hen readily synthesizes the cholesterol contained in her eggs, even when phytosterols are available in the feed. Schoenheimer and Breusch (1933) found that mice, on a diet of bread, synthesize as much cholesterol in a month as their bodies contained at the beginning of the experiment. Thus it is evident that formation of cholesterol in the animal body is possible, and sometimes occurs.

The discovery that cholesterol and phytosterol are not isomeric places a great objection to the idea that any cholesterol originates by an isomerization of vegetable sterols. It is now established that such a transformation would involve not merely the molecular rearrangement which was formerly postulated, but a reduction in the number of carbon atoms. Thus it appears that the conversion of plant sterols into animal sterols is improbable.

In this connection one recalls the early experiments of König and Schluckebier (1908), in which no phytosterol was found in the body fat, brain, liver, and bile of animals after long feeding with vegetable fat. Schoenheimer (1931) argues that if conversion were the rule, "every rabbit should show a marked atherosclerosis, because the quantity of plant sterols present in the usual feed of rabbits is approximately ten times as high as the dose of cholesterol which calls forth an atherosclerosis." Actually the feeding of enormous doses of sitosterol and other phytosterols over long periods to rabbits and other animals produced no pathological changes, and occasioned no rise in blood cholesterol and no storage of sitosterol.

The preceding reference is to a review of Schoenheimer's investigations on sterol metabolism, which is perhaps the most important single publication on this subject. It covers the series of publications by Schönheimer, v. Behring, Hummel, and Schindel (1930) and many other outstanding references. Schoenheimer goes on to say that although the foregoing experiments are almost a demonstration that sitosterol is not absorbed by the rabbit, there remains the possibility that it is absorbed, then rapidly changed to cholesterol and as such very rapidly excreted. If this were the case, cholesterol or one of its derivatives would appear in the feces, but actually the feces of rabbits and other animals fed a purely plant diet contained only the original plant sterols, unaltered qualitatively or quantitatively. This finding was substantiated by the observation that no phytosterol occurs in the lymph of the thoracic duct. The conclusion was made that plant sterols are not absorbed, at least not in amounts greater than undetectable traces.

The occurrence of "ergosterol" in animals, and the fact that it can be detected in minute amounts by the spectrograph led Schönheimer, v. Behring, and v. Gottberg (1932) to investigate its absorbability. They fed rats, mice, and rabbits purified ergosterol over long periods of time, and found that the body sterols of these animals contained no more ergosterol than is normal for their species. Thus it was shown, in disagreement with Page and Menschick (1930), that ergosterol, if absorbed, was not stored. They then fed dogs a mixture of cholesterol and ergosterol in equal parts, and found that the cholesterol of their thoracic lymph contained no more than the usual trace of ergosterol. To this evidence that ergosterol is not absorbed Schoenheimer (1931) adds the cautious comment: "One can, however, not exclude the possibility that vanishingly small but biologically important amounts of ergosterol may, nevertheless, be absorbed over long periods of time." Later Schönheimer and Dam (1932) and Menschick and Page (1932) reported that the laying hen does absorb small amounts of ergosterol. Thus it appears that in one instance, at least, the animal body is able to absorb ergosterol. That it does not do so readily seems fortunate, as otherwise unlimited amounts of vitamin D might be formed when the skin is exposed to sunlight.

Noting the facility with which the animal body absorbs vitamin D, which is stereoisomeric with plain ergosterol, Schoenheimer (1931) investigated the absorbability of other pairs of isomers. He found that allocholesterol, unlike cholesterol, was not readily absorbed. Moreover, none of the four isomeric dihydrocholesterols was absorbed. The comparison was also made with cholesterol and sitosterol, but this does not add much to the argument because these sterols are no longer regarded as isomers. "These observations then led to the conclusion that the organism behaves in an exceptionally specific manner in the absorption of the sterols. Even isomerism suffices to alter the absorbability and the saturation of the unsaturated bond changes the easily absorbable body to a completely unabsorbable form."

Schönheimer and Hummel (1931) investigated the absorption of cholesteryl esters by experiments with the oxalate. They found, after administering cholesteryl oxalate to mice, that cholesterol, but no oxalate, was stored in the liver. Oxalate appeared in the urine. It was evident, therefore, that the ester had undergone hydrolysis, presumably prior to absorption.

The two dihydrocholesterols which occur in feces, namely, coprosterol and $\beta$-cholestanol, may now be considered in relation to cholesterol. Flint (1862) knew only the former, and assumed it to originate from

cholesterol by digestive action. Bondzyński and Humnicki (1896) suggested that coprosterol results from a bacterial reduction of cholesterol. Dam has just demonstrated the reduction *in vitro*, according to word received as this goes ro press. Müller (1900) strongly supported the hypothesis of bacterial reduction in the gut, for he showed that a milk diet, which alters the bacterial flora, also inhibits the reduction to coprosterol, and permits cholesterol itself to appear in the feces. Schoenheimer, using improved analytical methods, found no coprosterol in the sterol of the tissues, *i.e.*, no evidence that coprosterol originates elsewhere than within the gut. Therefore its origin by the action of the anaerobic bacteria of the gut on cholesterol appears to be established.

β-cholestanol, present in small amounts with coprosterol in feces, has a different origin. Schoenheimer found that it is present in tissue sterol to the extent of 2 or 3 per cent. Since it is not absorbed, it must be formed in the body tissues. To account for the formation of this dihydrocholesterol in the tissues, he postulated a hydrogenation-dehydrogenation reaction, in which cholesterol undergoes reduction to β-cholestanol simultaneously with dehydrogenation to ergosterol. Unfortunately, this explanation is patently out of agreement with the subsequent discovery that ergosterol contains 28 carbon atoms, against 27 for cholesterol and its reduction products.

The route of excretion of β-cholestanol was investigated by Schoenheimer (1931). First he found that gallstone cholesterol is singularly poor in β-cholestanol. Thus it appeared unlikely that the bile is the route whereby this particular sterol reaches the feces. Schönheimer and Hrdina (1932) found that the secretions of the stomach and pancreas of dogs were practically devoid of sterols of any kind. On the other hand, the fresh intestinal secretion contained both cholesterol and β-cholestanol. It was argued that if the intestine behaves like the kidney, secreting primarily a large volume of fluid, the larger part of which is reabsorbed, then one should be able to demonstrate the accumulation of the non-absorbable β-cholestanol in blind loops of sterile gut.

Schönheimer and v. Behring (1930) isolated the lower gut of dogs, and found after one or two months that it contained sterile masses of concentrated, but presumably normal intestinal secretion, comparatively free from absorbable materials such as cholesterol. Coprosterol was absent, but β-cholestanol occurred in very significant amounts. Schönheimer and Hrdina (1932) obtained similar findings, using blind loops of the small intestine. They cited also Boehm's (1911) report on a woman who, following a surgical accident, accumulated several

grams of β-cholestanol in a loop of intestine. Thus the route of excretion of β-cholestanol was found to be through the intestinal wall.

It is clear that the body does not eliminate much cholesterol as such. Except for that which is lost in the desquamation of skin, or secreted in milk, the greater part is reduced, and leaves the gut as coprosterol and β-cholestanol. A little may be conjugated and pass out in the urine. Some is probably completely destroyed.

*Physiological destruction.* Although the literature abounds with references to the storage and excretion of absorbable sterols, it is only recently that their destruction in the body has been demonstrated. Dam (1931) observed a loss of sterol in young chickens which could not be accounted for on the basis of excretion. Page and Menschick (1932) fed moderately large amounts of cholesterol to rabbits in the course of balance experiments. Sterol was deposited throughout the body excepting the brain. After all depots were accounted for, including the fur and excreta, there was indicated a destruction amounting to over a gram per week. Menschick and Page (1933) performed similar balance experiments with the cat, an animal which, unlike the rabbit, normally takes cholesterol in its food. The destruction of cholesterol was again evidenced. Schönheimer (1933) observed the destruction in a human freak who could absorb, but not excrete sterols. Schoenheimer and Breusch (1933), in a beautiful experiment with mice, found that these animals which, on diets low in cholesterol, can synthesize their body content of cholesterol in a month, can also destroy five times this amount on diets rich in cholesterol. Fat in the diet had no effect on the cholesterol balance. The authors concluded "*that in the tissues cholesterol is continually being formed and destroyed. Either a positive or negative balance may be found depending upon experimental conditions, i.e. synthesis may be in excess of destruction or vice versa.*"

It is appropriate at this point to mention the enzyme, or enzymes, known as cholesterase (cholesterol esterase). The existence, but not the function, of this enzyme has been clearly demonstrated by Schultz (1912), Cytronberg (1912), Porter (1916), Thannhauser (1923), Nomura (1924), Shope (1928), and others. Cholesterase apparently occurs widely in the organs, tissues and fluids of man and animals. Presumably its rôle is confined to the hydrolysis of cholesterol esters in digestion, and perhaps to their re-synthesis after absorption. So far as is known, it plays no part in the rupture of the sterol ring.

In nature the decay of vegetation involves an enormous disintegration of sterol. Obviously the sterol undergoes rapid resolution, for otherwise

accumulations of it would soon cover the earth. It has been suggested that some of the polycyclic components of petroleum and coal originated in the sterols of ancient plants and animals. Bacterial action presumably accounts for the loss of sterol in natural decay, although the disintegration of sterols by bacteria has not been demonstrated in the laboratory.

*Formation of gallstones.* It is trite to say that cholelithiasis is fundamentally the result of a fault in cholesterol metabolism. Chevreul (1824) was the first clearly to recognize that the cholesterol contained in the bile provides the substance of gallstones. Gobley (1846), commenting on the solubility of cholesterol in soap solutions, suggested that the separation of cholesterol from the bile to form stones is the result of a deficiency of "soap." The innumerable studies since Gobley have contributed surprisingly little to a clear understanding of the fundamental causes of gallstones.

The recent work of Schoenheimer and Hrdina (1931) and Andrews, Schoenheimer, and Hrdina (1931a, b, 1932) is, however, significant. They showed that most of the cholesterol of human bile is present in the form of water-soluble complexes with the bile salts (*cf.* Gobley's soap). These complexes are so unstable that when they are subjected to dialysis the bile salts pass out and the cholesterol is precipitated. Therefore it would appear that any pathological change which lowers the ratio of bile salt to cholesterol could cause precipitation. Now, obviously, the healthy gall bladder mucosa is constantly absorbing water, bile salts, and cholesterol at the normal rate and ratio. Experimentally it was found that a bladder rendered abnormal by infection or trauma is more permeable to bile salts than to cholesterol. Moreover, analysis showed that the bile in cholelithiasis has a much lower bile salt-cholesterol ratio than bile from bladders not containing stones. Thus the theory was evolved and supported that "cholesterol stones are due to a faulty differential absorption of bile acids and cholesterol by the abnormal gall bladder mucosa."

With this as an explanation of the immediate mechanism of gallstone formation, the immense medical literature on the subject may be regarded as a contribution to the understanding of the ultimate causes. No attempt to survey the larger field can be made here, but the reader's attention is called to the latest brief review, by Patey (1933).

FUNCTION OF THE STEROLS. The investigation on the distribution of cholesterol in the animal kingdom by Dorée (1909) was carried out with the idea that unless cholesterol is of primary importance to life, there

should be animals in which it is absent. None such was found. Inferentially, from their ubiquitous presence, the sterols are considered to have important functions, but in spite of the numerous rôles which have now and again been ascribed to them, the only function which has been actually demonstrated is the part played as the acceptor of ultraviolet radiations. Shope (1928), commenting on the wide occurrence of cholesterol esterase, says that "in view of the complete absence of knowledge concerning the function of cholesterol itself," any surmises regarding the enzyme would be unfruitful at this time.

The following suggested rôles seem important enough to be mentioned in review. By force of repeated citation, some of these have come to be regarded as established, but the facts do not warrant such categorical conclusions.

1. A physiological relation between the sterols and the physiologically important substances which are chemically related to them is a possibility of intriguing interest. Yet even with the bile acids, no *physiological* relation has been established (Thannhauser, 1923; Smith and Whipple, 1928).

2. The occurrence of sterols in the skin, in wool grease, and in the preen gland of birds suggests that they exert a conditioning action on the skin, hair, and feathers. The cholesterol content of human skin fat amounts to about 19 per cent, according to Eckstein and Wile (1926). One recalls the cosmetic value of lanolin, and the damage to wool resulting from its removal.

3. That cholesterol functions as a conveyer in the absorption of fats is one possible interpretation of certain metabolism experiments. Thus, Wacker and Hueck (1913) observed a great increase in cholesterol ester in the blood after feeding free cholesterol. Mueller (1915b) noted that during absorption, free cholesterol is partly esterified, while cholesterol ester, on the other hand, is partly hydrolyzed by the time it reaches the chyle. Bloor (1916a, b) noted a remarkable parallelism between the cholesterol content of blood and the fatty acid content, even in severe lipemias. Knudson (1917) fed olive oil, which contains no absorbable sterol, to dogs, and found a rise in the ratio of cholesterol esters to free cholesterol in the blood. Thannhauser (1923) noted that cholesterol is absorbed only when fat is present. Thannhauser called attention to the fact that the cholesterol of human bile exists only in the "free" state, *i.e.*, not as ester.

4. From a consideration of the abundance of cholesterol in the brain and nerves, its high dielectric constant, and its variation in the blood

with mental disease, Minovici (1927) concluded that it functions as an insulating medium for the myelin sheaths. A measure of support for this hypothesis is found in several old analytical determinations of the cholesterol content of the brain in relation to age, and particularly the observation by Page and Menschick (1931) that in man the cholesterol content of the brain is lowest in fetal life, 0.5 per cent. After birth it gradually rises, and the rise continues in childhood. Adult brains (age 36, 72, and 81 years) each showed 1.9 per cent, a figure in fair agreement with Beneke's (1880) value of 2.2 per cent. (The ergosterol content, at most a trace, moves in reverse order, decreasing with age.)

5. In the theory of cellular permeability and membrane equilibrium, advanced by Overton (1900, 1901) and supported by Pascucci (1905), every living cell is supposed to be limited by a membrane relatively rich in phosphatides and sterol. Significance is attached to the fact that the hemolysis which occurs when water is injected in the blood does not occur if cholesterol is injected simultaneously. Narcosis is associated with the solvent action of narcotic substances on cholesterol. In line with Overton's theory, Mathews (1913) suggested that the great instability of starfish eggs, compared with sea urchin eggs, is due to their relative paucity in cholesterol.

6. In the discussion of distribution it was shown that sterols are present in seeds, pollen, spores, ova, and sperms. This may indicate some peculiar need for sterol on the part of the developing embryo. In one particular instance, the egg, Mueller (1915a) has interpreted the abundance of cholesterol in relation to the calcification of the embryo. During the third week of incubation there is a large increase in cholesterol ester, at the expense of free cholesterol. It was assumed that the phosphorus required for calcification comes from the breaking down of lecithin, and that the fatty acids liberated at the same time combine with the cholesterol, the esterification thus being a special detoxifying mechanism.

7. A possible rôle of the sterols, too frequently declared a fact, is that of neutralizing toxins. Phisalix (1897) first observed that cholesterol is one of the bile constituents which exerts the detoxifying action against snake venom. Ransom (1901) added the observation that cholesterol inhibits hemolysis by saponin. Hédon (1900–1901) observed that hemolytic glucosides detoxified by cholesterol again exhibited toxicity when injected into the animal, an effect attributed to dissociation or to poisoning of nerve cells. Hausmann (1905) investigated the mechanism of the detoxification of saponin, finding that both the hydroxyl

group and the double bond of the sterol are involved. Engagement of the hydroxyl group by esterification destroyed the effect; saturation of the double bond merely lessened it. Not only cholesterol, but various phytosterols, were effective. Abderhalden and Le Count (1906) found that cholesterol, but not its esters, opposed the lytic action of saponin, solanin, cobra venom, and tetanus toxin.

Windaus (1908, 1909) reviewed some of the early investigations on the antihemolytic effect, and determined that the sterols form definite addition products with the substances which they detoxify. Digitonin, in particular, forms such a stable complex, that it has become the outstanding analytical reagent for the precipitation of sterols and for the separation of sterols from their esters. It was with digitonin that Windaus (1916) demonstrated one of the remarkably specific relations between chemical structure and physiological action of the sterols. He found that β-cholestanol inhibits hemolysis by digitonin, whereas the stereoisomeric ε-cholestanol is almost without action. The β-isomer forms an insoluble digitonide, whereas the ε-isomer does not.

The number of investigations on the detoxifying action of sterols is large. In passing, it will suffice to mention Kyes (1903), Pascucci (1905), Madsen and Noguchi (1905), Pribram (1906), Morgenroth and Reicher (1907), Delezenne and Ledebt (1911), Mayer and Schaeffer (1912), Levene and Rolf (1923), Roffo (1925), and Lumière and Grange (1930a). Additional references are given in the review by Minovici (1927). In substance, these investigations have shown that numerous hemolytic agents—venoms, saponins, bacterial toxins, bile salts, soaps, hexylresorcinol, etc., are neutralized by sterols. The effect can be demonstrated either *in vitro* or *in vivo*.

The claim that sterols normally function as detoxifying agents must be regarded as largely presumptive, supported mainly as follows. *a*. The sterols do exhibit antihemolytic powers, albeit against substances which the body does not normally encounter. *b*. In view of the action against tetanus toxin it may be significant that in most febrile infectious diseases blood cholesterol is reduced at the height of the disease (Minovici, 1927; Peters and Van Slyke, 1931). This is especially noticeable in tuberculosis (Sweany, Weathers, and McCluskey, 1924). *c*. In the case of cobra venom, the lytic substance against which the sterols react is lysolecithin or lysocephalin, derived from lecithin or cephalin by the loss of the unsaturated fatty acid radicals. Significance can therefore be attached to the simultaneous occurrence of cholesterol and lecithin, and to the fact that the two maintain a fairly constant ratio. *d*. Minovici

(1927) mentions the occurrence of cholesterol in leucocytes in relation to the theory of phagocytosis. After Hürthle (1896) discovered cholesterol esters in blood, Hepner (1899) reported that the esters are confined to the plasma. This was disputed by Cytronberg (1912), but confirmed by Bloor and Knudson (1917), Richter-Quittner (1920), Gardner and Gainsborough (1927a), and others. Apparently the corpuscles, in the post-absorption period, contain practically all of their sterol in the free state, which is the form capable of exerting the antihemolytic action, and indeed the leucocytes contain five times as much as the red cells (Wacker and Hueck, 1913). *e.* The low blood cholesterol characteristic of anemia may reflect either a cause or an effect: There may be insufficient sterol to prevent hemolysis, or there may be a hemolytic substance present in excess in this condition (Bloor, 1916a).

8. A protective action of cholesterol against anaphylactic shock is suggested by the experiments of Lumière and Grange (1930b). According to Lumière's theory, anaphylactic shock is the result of a flocculation in the blood stream, for it can be produced by the injection of such materials as Chinese ink or barium sulphate suspended in serum. The effect was inhibited by cholesterolized serum.

ACTION OF LIGHT ON STEROLS—FORMATION OF VITAMIN D. *Discovery of activation.* The researches which culminated in the activation of ergosterol were, in a sense, a bringing together of old observations on the therapeutic value of fish oils and of light. Remedial virtues had been associated with these agencies for so many years that, in retrospect, it may seem remarkable that the discoveries of the present decade were not made earlier. Thompson (1854), writing on the anemia of tuberculosis, stated that "the use of almond-oil and of olive-oil was not followed by any remedial effect, but from the cocoa-nut oil results were obtained almost as decided as from the oil of the liver of the Cod. . . . ." His preparation was undoubtedly made from sun-dried copra, for it was "a pure cocoa oleine, obtained by pressure from crude cocoa-nut oil, as expressed in Ceylon and the Malabar coast from the *Copperah* or dried cocoa-nut kernel. . . . ." Thus the first report on the therapeutic use of an irradiated substance came to naught for want of an interpretation.

Ritter (1902) and Lewkowitsch (1909) noted that cholesterol slowly decomposes under the action of light and air. The light-sensitivity of cholesterol, and especially of ergosterol, was observed by Schulze and Winterstein in 1904 and again in 1906. Lifschütz (1922) noted that the decomposition of cholesterol by sunlight occurred either in the dry state or in alcoholic solution. Schulze and Winterstein (1906) reported that

prolonged exposure resulted in a lowering of melting point and the formation of a resin. From the fact that the action was retarded in an atmosphere of carbon dioxide they assumed that it was essentially an oxidation.

Sunshine, like fish oil, is an old remedy for rickets. Its importance, however, was not fully appreciated until Huldschinsky (1919, 1920), by means of radiographs, clearly demonstrated the healing action of sunlight and of the light from the quartz mercury arc. Hess, Pappenheimer, and Weinstock (1922) determined that the effective wavelengths of light are the shorter ultraviolet waves of the solar spectrum, or the still shorter waves of artificial sources. Goldblatt and Soames (1923) discovered that the livers of irradiated rats, when fed to non-irradiated rats, convey some of the virtue of irradiation to the animals which eat them.

This, in greatest brevity, is the background for the discovery of the activation of foods and sterols by irradiation. Several thousand references to papers covering experimental rickets, and the chemistry and physiology of vitamin D, are given in the following specialized reviews: Park (1923), McCollum and Simmonds (1929), Hess (1929), MacLeod (1930), Blunt and Cowan (1930), Goldblatt (1931), Browning (1931), Medical Research Council (1932), Laurens (1933), Weidlich (1933), Tanret (1933), and Nicola (1934).

In 1924 Hess and Steenbock independently and almost simultaneously announced the discovery that exposure of edible materials to ultraviolet light endows them with antiricketic activity. Hess found that inert oils, namely, cottonseed and linseed, acquired this property of cod liver oil. Steenbock found the same to be true of muscle tissue, mixed feed, and fats.

A succession of publications by Steenbock and by Hess rapidly expanded the knowledge of activation. The original brief communications were published *in extenso* by Steenbock and Black (1924), Steenbock and Nelson (1924), and Hess and Weinstock (1924c). These papers, together with several supplementary contributions and the Steenbock patent of 1928, revealed that edible materials in great variety are activatable, and that activation depends upon the same short wavelengths which are effective directly in the cure of rickets. Furthermore, it appeared that activation is relatively permanent, and that it is not a phenomenon of oxidation. The active moiety of irradiated linseed oil was traced to the unsaponifiable fraction, just as in the case of cod liver oil itself.

These early publications contained some technical faults, such as the inclusion of test animals which lost considerable weight during the period of assay. A few erroneous or doubtful conclusions were drawn, but the majority of the findings, including those presented above, have withstood the test of repetition.

Shortly a second stage in the knowledge of activation was reached, when it was demonstrated that sterols become antiricketic upon irradiation. Hess, Weinstock, and Helman (1925) succeeded in activating phytosterol and cholesterol, and to a lesser extent, lanolin. Steenbock and Black (1925) found that cholesterol which had been specially "purified" by esterification still became antiricketic upon irradiation, but that phytosterols from old vegetable oils acquired only slight, if any, activity. Rosenheim and Webster (1925) activated cholesterol.

Thus it became evident that in foodstuffs the sterol fraction contains the acceptor of the activating rays. The course of investigation now turned to the elucidation of the chemical changes induced by irradiation in the sterols.

Hess and Weinstock (1925a, b) introduced the use of the quartz spectrograph for investigating the chemistry of activation. They found that ordinary cholesterol was somewhat opaque to ultraviolet light, and that irradiation decreased its opacity. On the other hand, dihydrocholesterol and dihydrositosterol, which were not activatable, were practically transparent, and the transmission of ultraviolet light was not altered by irradiation. Unfortunately, a mercury arc was used as the light source for the spectrograms. Since this gives a discontinuous spectrum, nothing could be learned of the spectral structure in the region of absorption.

Schlutz and Morse (1925), with better technique, determined that the absorption spectrum of ordinary cholesterol is banded. Their densitometer tracings showed two maxima of absorption at approximately 294 and 283 m$\mu$. A trained observer, biased, perhaps, by later knowledge, can see in these original tracings two additional faint inflections. In retrospect it thus appears that Schlutz and Morse were the first to record what is now recognized as the provitamin absorption spectrum. They noticed that after brief irradiation the inflections gave way to general absorption, and, considering Beer's law, they postulated that either the cholesterol had been at least half metamorphosed, or else "*the substance in which the absorption spectrum is changed may be a small amount of impurity in the cholesterol which is not removed by repeated crystallizations from alcohol, and which is exceedingly absorptive.*"

Such are the coincidences of research that, on December 10, 1926, reports from three separate laboratories confirmed the contamination hypothesis. Rosenheim and Webster (1926b) found that cholesterol which had been regenerated from cholesterol dibromide was so pure that it no longer showed the characteristic absorption spectrum, and was no longer activatable. They had previously noted (1926a) that ergosterol is activatable, but having made no quantitative determination of the degree of activatability, they did not now recognize "ergosterol" as the contaminant removed by bromination.

Heilbron, Kamm, and Morton (1926) reported that fractional crystallization of cholesterol led to the accumulation, in the least soluble fraction, of the substance responsible for the characteristic absorption spectrum. They recognized a third absorption maximum, 269 mμ. Irradiation destroyed the three bands, leaving only general absorption.

Pohl (1926), by the technique of photoelectric photometry, also detected three absorption bands in cholesterol, which faded upon irradiation. With the knowledge that complete disappearance of the bands corresponded to the destruction of only a trivial fraction of the cholesterol, he concluded that the absorbing substance was present only in minute amount. He found that cholesterol purified *via* the dibromide, or *via* allocholesterol, and sitosterol purified *via* the dibromide, did not show the usual absorption.

These simultaneously announced studies left no doubt that an impurity present in ordinary cholesterol and phytosterol is responsible for at least the greater part of the antiricketic activity conferred upon these sterols by irradiation.

After an interchange of suggestions between the investigators, the impurity, or provitamin, was presently identified as ergosterol. Pohl (1927) found that the three known absorption bands of ordinary cholesterol are also exhibited by ergosterol. Moreover, ergosterol showed them in vastly greater intensity than cholesterol. Chemical studies and a biological assay were announced by Windaus and Hess (1927). In keeping with expectations, the antiricketic potency of irradiated ergosterol was found to be far greater than that of irradiated cholesterol. At the same time, Rosenheim and Webster (1927a, c) reported essentially the same findings, from which they concluded that the precursor of vitamin D is ergosterol, or a sterol of similar constitution.

Bills, Honeywell and MacNair (1928) confirmed these remarkable discoveries in a way which seemed to leave no reasonable doubt that ergosterol is the contaminant provitamin. By the use of a hydrogen

discharge tube, which emits an exceptionally continuous ultraviolet spectrum, they discovered that ordinary cholesterol exhibits a fourth absorption band, λ 260 mμ. Exactly the same band was shown by ergosterol, making the series λλ 293.5, 282, 270, and 260 mμ with four points of identity instead of three. An indication that the contaminant was ergosterol, and not some sterol with the same absorption bands, was obtained by comparing the rates of destruction. Acetone solutions of ordinary cholesterol, and of ergosterol-free cholesterol plus added ergosterol, were boiled with potassium permanganate. The absorption bands, identical in intensity at the outset, faded at essentially the same rates.

*Note added in press.* Despite the convincing evidence that ergosterol is the provitamin in cholesterol, Waddell (1934) has just published experiments which indicate that such is not the case. He found that irradiated cholesterol was many times more effective on chickens, rat unit for rat unit, than ergosterol which had been irradiated either by itself or in the presence of cholesterol. Waddell's experiments contain no obvious flaw, and if confirmed, will necessitate revision of the provitamin concept.

*Physical chemistry of activation.* Morton, Heilbron and Kamm (1927) reported that the disappearance of ergosterol irradiated in dilute solution bore a linear relation to the time. Unpublished data from this laboratory show, however, that concentrated solutions do not exhibit the linear relationship. It is probably true, and certainly logical to expect, that under no conditions is the time-disappearance curve actually a straight line, for the products of irradiation are numerous and some of them are sufficiently absorptive to act as filters against the remaining unchanged ergosterol.

The wavelengths which affect ergosterol are, of course, those which it absorbs. Ergosterol absorbs strongly from 305 mμ to 230 mμ in the far ultraviolet region (Cox and Bills, 1930). But it exhibits a little absorptive power beyond these limits in both directions. Wavelengths as long as 313 mμ are known to activate it slightly, and there is reason to believe that still longer waves play a part in the decomposition of ergosterol and/or of its primary irradiation products (Lahousse and Gonnard, 1929). The predominance of these longer waves may explain the relatively low efficiency of sunlight in activation. In the experiments of Windaus, Borgeaud, and Brunken (1927), Windaus and Borgeaud (1928), and Windaus and Brunken (1928) white light in the presence of optical sensitizers resulted in definite chemical changes in ergosterol, but not in the formation of vitamin D. Cathode rays (Knudson and C. N. Moore, 1929) and radium emanation (R. B. Moore

and DeVries, 1931) have a moderately effective activating action on ergosterol. X-rays do not activate (Goldblatt, 1931). The few published reports to the contrary are not supported with acceptable bioassays. Radio waves of high intensity and short wavelength are without action (unpublished investigation).

The early work of Pohl (1926) suggested that activation may involve the addition of energy to the sterol molecule through an electron displacement. Measurement of the energy relations was attempted by Kon, Daniels and Steenbock (1928), who ventured a definite computation in ergs. In a similar undertaking, Marshall and Knudson (1930) recognized that the measurement of the energy required for activation is complicated by the occurrence of secondary reactions. Bills, McDonald, BeMiller, Steel and Nussmeier (1931) considered that if the energy of activation remains resident in the activated molecule, a determination of the heat of combustion should reveal it. Actually they found no difference in this respect between plain ergosterol and a very potent irradiation product containing over 20,000 international units of vitamin D per milligram. It is interesting to observe that provitamin D, which is as important to animals as chlorophyll is to plants in the utilization of light, does not play even a small rôle in the storage of energy.

The researches of Kon, Daniels and Steenbock (1928), Webster and Bourdillon (1928), and Marshall and Knudson (1930) seemed to show that vitamin D is formed with equal efficiency by any wavelengths within the region of the principal absorption of ergosterol. Strictly with reference to the formation of vitamin, and apart from side reactions and decompositions, such a conclusion may be correct. It is known, however, that the irradiation of ergosterol produces several substances in succession and/or in parallel. This fact was established by the early investigations of Rosenheim and Webster (1927b), Webster and Bourdillon (1928), Smakula (1928), Bills, Honeywell and Cox (1928), Fabre and Simonnet (1929), Webster and Bourdillon (1929), Delaplace and Rebière (1929), Bourdillon, Fischmann, Jenkins and Webster (1929), Windaus, Westphal, v. Werder and Rygh (1929), Lahousse and Gonnard (1929), Reerink and van Wijk (1929), and others. It has been confirmed in many later investigations. Inasmuch as the several products of irradiation have absorption spectra different from ergosterol, they are themselves susceptible to different wavelengths. The net result is that the sequence of products obtained by irradiation varies with the wavelengths of light employed (Reerink and van Wijk, 1929; Windaus, 1930; Askew, Bourdillon, Bruce, Jenkins and Webster, 1930b).

The temperature coefficient of activation is small. Bills and Brickwedde (1928) found that cholesterol containing 1.2 parts per 1000 of "ergosterol" was readily activated at −183°, although the product was somewhat less potent than the product of similar irradiation at room temperature. Webster and Bourdillon (1928) irradiated ergosterol at temperatures between −195° and +78°, and again observed the smallness of the temperature coefficient. Since bimolecular reactions have a large temperature coefficient, and are generally inhibited at very low temperatures, the conclusion from these experiments was that the change involved is monomolecular. All subsequent experience, including the analysis of crystalline preparations of vitamin D, has borne out the view that activation consists in an isomerization of the ergosterol molecule.

The composition of the irradiation product is influenced by the purity and nature of the solvent in which the ergosterol is dissolved. Ergosterol in the dry state takes activation poorly, presumably because the first irradiation products which form on the exposed surfaces act as filters to prevent light from reaching the under layers until the surface products are destroyed. This principle may explain the observation of Windaus, Westphal, v. Werder and Rygh (1929) that agitation of the solution during exposure enhances the formation of vitamin D. To the extent that the solvent itself is opaque to short waves, it necessarily exerts a filtering action on the activating rays.

The presence of dissolved oxygen in the solvent markedly affects the spectral picture of activation (Smakula, 1928; Bills, Honeywell and Cox, 1928; Reerink and van Wijk, 1929). It does so by altering the by-products of activation, rather than by affecting to any large extent the formation or destruction of vitamin D (Bills, Honeywell and Cox, 1928). The by-products which form by oxidation make difficult the crystallization of the vitamin (Angus, Askew, Bourdillon, Bruce, Callow, Fischmann, Philpot and Webster, 1931). In the experiments of Beard, Burk, Thompson and Goldblatt (1932) extreme freedom from oxygen in a dry activation did not increase the antiricketic potency of the product. As is usual in the absence of a solvent, the potency attained was only moderate, amounting in this case to roughly one-tenth of the value expected when activation is performed in ether. The investigations of Windaus (1930), Angus, Askew, Bourdillon, Bruce, Callow, Fischmann, Philpot and Webster (1931) and Bills, McDonald, BeMiller, Steel and Nussmeier (1931) show that vitamin D, either in crystalline form or as a resin, is decidedly more stable to oxidation than some of the

non-vitamin substances which are formed with it in irradiation. Its stability is about the same as that of ergosterol itself.

Apart from any light-filtering action of solvents, and from any rôle which they may play as carriers of dissolved oxygen, there appears to be what Bills, Honeywell and Cox (1931) termed a specific solvent effect on activation. This was revealed by parallel spectrographic and biologic examinations on the course of activation in alcohol, cyclohexane, and ether. The importance of the specific solvent effect was shown by the fact that the time required for the attainment of maximum potency in ether was longer than the time required for the entire sequence of activation and destruction in alcohol. The maximum potency reached in ether was higher than in alcohol or in cyclohexane, but the spectral changes were most conspicuous in alcohol.

*Individual products of irradiation.* Attempts to characterize the individual products of irradiation, and to ascertain the number of them by interpretation of absorption spectra have been numerous but not very profitable. The reason is easily understood, for with mixtures of substances having overlapping absorption bands, determinations of the position and height of the bands are unreliable. Precision methods of bioassay and chemical methods of isolation have been the principal means of progress during the later stages of the study.

The preparation of the starting material, ergosterol, in high purity, has been described by Bills and Honeywell (1928), Callow (1931a), and others. The chemical and physical constants of nearly anhydrous ergosterol are approximately as follows: $C_{28}H_{43}OH$; $|\bar{}_3$; m.p. 166°; λλ 260, 270, 282, 293.5 mμ; $[\alpha]^{20}_{5461} = -171°$; $[\alpha]^{20}_{D} = -132°$ in chloroform. Ergosterol is crystalline, but the irradiation-product, after removal of unchanged ergosterol, is a nearly colorless resin. The isolation of several of its components is briefly recounted in the following paragraphs.

*1.* Vitamin D. Calciferol. An ingenious method for the distillation and fractional condensation of the resin in a high vacuum was described by Askew, Bourdillon, Bruce, Jenkins, and Webster (1930a). Small amounts of potent crystals were obtained. The method was improved, and a detailed study of the active crystals was reported by Angus, Askew, Bourdillon, Bruce, Callow, Fischmann, Philpot, and Webster (1931). The name, calciferol, was given to the crystalline substance. A few months later Askew, Bourdillon, Bruce, Callow, Philpot, and Webster (1932) discovered that the original calciferol was a mixture, separable *via* the 3.5-dinitrobenzoate into pure calciferol and two in-

active sterols. One of the latter, pyrocalciferol, was merely a thermal transformation-product, produced during the distillation. The other, "sterol X", had apparently come over from the original irradiation-product. By the new esterification method calciferol could be obtained directly from the resin, without distillation.

Pure calciferol melts at 115–117°. In alcoholic solution it has $[\alpha]^{20}_{5461} = +122°$ and $[\alpha]^{20}_{D} = +102.5°$. It shows strong ultraviolet absorption, with a maximum at 265 mμ. Analyses indicate that it is isomeric with ergosterol, $C_{28}H_{43}OH$, $|^{-}_{3}$. Its antiricketic potency amounts to 40,000 I.U. per mgm. Like all other products of irradiation, it does not give a precipitate with digitonin.

While the isolation of calciferol was in progress in England, Windaus and his associates in Germany were developing a different technique to the same end. Windaus (1931b) and Windaus, Lüttringhaus, and Deppe (1931) reviewed their preliminary studies on activation, and announced that a crystalline vitamin D was obtained by removing the inactive components of the resin by means of maleic or citraconic anhydride. Soon after this, Linsert (1931) obtained another crystalline preparation somewhat different in its properties from the first. For a few weeks Windaus and Lüttringhaus (1931) regarded these two preparations as distinct forms of vitamin D, and called them vitamin $D_1$ and vitamin $D_2$ respectively. Presently, however, Windaus, Linsert, Lüttringhaus, and Weidlich (1932) discovered that the Linsert preparation was essentially identical with Bourdillon's pure calciferol, while the first German preparation was an addition compound of calciferol with an inactive sterol.

*2.* Lumisterol. $C_{28}H_{43}OH$; $|^{-}_{3}$; m.p. 118°; λλ 265, 280 mμ; $[\alpha]^{19}_{5461} = +235.4°$; $[\alpha]^{19}_{D} = +191.5°$ in acetone.

The sterol which was removed from the original impure calciferol preparations was called sterol X by the English workers, and given the name, *Lumisterin*, by the Germans. It was studied in considerable detail by Windaus, Dithmar, and Fernholz (1932), who obtained it by the fractional crystallization of the acetate of the lumisterol-calciferol addition compound. The absorption spectrum of lumisterol is similar to that of ergosterol in range and intensity, but it shows only two maxima, instead of four. Like ergosterol, lumisterol gives the Rosenheim (1929) color reaction for the $\Delta^{1,2}$ double linkage in the sterol ring. Lumisterol appears to be the initial product of the irradiation of ergosterol. It probably is devoid of antiricketic action, but it is converted by irradiation into vitamin D. It forms with calciferol a definite addi-

tion compound in the ratio of 1:1, this being the vitamin $D_1$ of the German workers.

*3.* Tachysterol. $C_{28}H_{43}OH$; m.p. indeterminate; principal absorption band at 280 m$\mu$ and lesser bands at 268 and 294 m$\mu$; $[\alpha]_{5461} = -86.3°$; $[\alpha]_D^{18} = -70°$ in "*Normalbenzin.*"

The sterol which was removed from crude irradiation-product by citraconic anhydride was called *Tachysterin* by Windaus, Lüttringhaus, and Busse (1932). It was the object of a special study by Windaus, v. Werder, and Lüttringhaus (1932), who prepared it from the addition compound, tachysteryl acetate-citraconic anhydride, *via* tachysteryl 3.5-dinitro-4-methyl benzoate. These products form good crystals, although tachysterol itself does not crystallize. The absorption spectrum of tachysterol is characterized by its great intensity at 280 m$\mu$. Tachysterol is a source of trouble in the separation of irradiation products. Besides failing to crystallize, it exhibits an exceptional affinity for oxygen, so great that perbenzoic acid titrations to determine its degree of unsaturation have been uninformative. Tachysterol follows lumisterol and precedes calciferol in the sequence of irradiation products. It probably is devoid of antiricketic action, but it may have a slight toxic effect.

*4.* Toxisterol. Substance 248. Morton, Heilbron, and Kamm (1927), extending the work of Pohl (1927), showed that when ergosterol is irradiated, the three bands with maxima at 293.5, 282, and 270 m$\mu$ disappear, while a single new band of great intensity develops at 247 or 248 m$\mu$, and in turn fades away to weak general absorption in the far ultraviolet region. With inadequate biological evidence, the assumption was easily made that the new band represented vitamin D. Smakula (1928) concluded that substance 248 was not the vitamin. Bills, Honeywell, and Cox (1928), introducing precision methods of assay, showed that the *appearance of the band at 248 m$\mu$ coincided not with the development, but with the destruction, of antiricketic potency.* They, and also van Wijk and Reerink (1928) associated substance 248 with isoergosterol. Cox and Bills (1930) contributed further evidence of relationship to the isoergosterols, but noted a point of difference, namely, that substance 248 does not precipitate with digitonin.

Substance 248 has not yet been isolated. Bills, Honeywell and Cox (1928) found that irradiation products withdrawn for examination at the moment when substance 248 was at its maximum concentration, still contained some vitamin D. Further irradiation totally eliminated antiricketic activity, while only a small amount of substance 248 was

destroyed. Laquer and Linsert (1933) attributed a toxic quality to substance 248, and proposed the name, *Toxisterin* (toxisterol). The product which they investigated still contained some vitamin D, but not enough to account for the relatively great toxicity. It was evidently a mixture similar to that which Hoyle (1930b) obtained by *limited* over-irradiation, and which he reported to have toxic-calcifying properties all out of proportion to the antiricketic potency. Such a mixture, unfortunately, seems to have been the "vitamin D" of the I.G. Farbenindustrie Aktiengesellschaft patent of 1928 (the old Vigantol of toxic repute). There is considerable evidence, both spectrographic and toxicologic, that toxisterol is formed most readily when alcohol is the solvent in which the ergosterol is irradiated (Bills, Honeywell and Cox, 1928, 1931; van Wijk and Reerink, 1928; Dixon and Hoyle, 1928; Harris and Moore, 1929; Hoyle and Buckland, 1929; Hoyle, 1930b; Kern, Montgomery and Still, 1931).

*5*. Suprasterol I. $C_{28}H_{43}OH$; $|_3^=$; m.p. 104°; general absorption below 250 m$\mu$; $[\alpha]_D^{18} = -76°$ in chloroform.

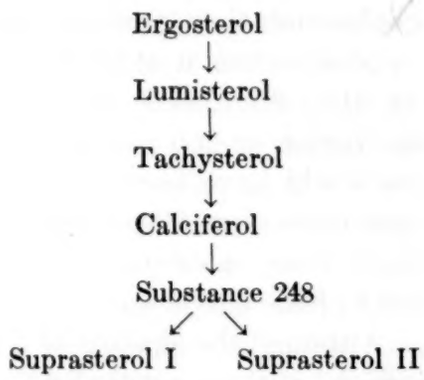
*6*. Suprasterol II. $C_{28}H_{43}OH$; $|_3^=$; m.p. 110°; general absorption below 250 m$\mu$; $[\alpha]_D^{19} = +62.9°$ in chloroform.

The products of extreme over-irradiation, presumably the end products of the series, were designated *Suprasterine* by Windaus, Gaede, Köser, and Stein (1930). They recognized two isomers, suprasterol I and suprasterol II, which they separated by taking advantage of differences in the solubility of the allophanic acid esters. The suprasterols are not antiricketic and are only slightly toxic. They give the Rosenheim reaction in modified form.

A general survey of the irradiation products, with absorption curves on a common basis, was given by Windaus, Lüttringhaus, and Busse (1932). Setz (1933) has brought the genetic relationships of the products up to date, and confirmed the view that the changes involved are not reversible. It appears from his work that when ergosterol is irradiated for not too long a period, the product produced by wavelengths below 280 m$\mu$ is chiefly tachysterol and calciferol, whereas the product of longer wavelengths is chiefly lumisterol and calciferol.

The genetic series is probably best summarized in the following diagram. This is based mainly on the papers by Windaus and his colleagues, particularly Setz. There is some evidence that the scheme is not complete. Between lumisterol and tachysterol a protachysterol may occur. The reviewer has assumed that substance 248 precedes the suprasterols, because the absorption spectrum of over-irradiated

substance 248 (Bills, Honeywell and Cox, 1928) is essentially that of the suprasterols studied by the German workers. The name, calciferol, is retained for that form of vitamin D which has been isolated. It is not impossible that at least one other form of the vitamin occurs in crude irradiation-product.




*The several forms of vitamin D.* A year before the discovery of activated ergosterol, Bills (1926) reported that fuller's earth, acting on cholesterol, gave rise to a resin which had slight, yet definite action in the healing of rickets. Bills and McDonald (1926) showed that the antiricketic component of this resin was chemically different from that occurring in fish oils or that developed by irradiation. In a later study (1927) they found it to be a degradation product of cholesteryl ether. This work was confirmed by Kon, Daniels and Steenbock (1928), but it has remained only of theoretical interest. It showed, however, that antiricketic action is associated with more than one molecular configuration in the sterols.

With the discovery of ergosterol as provitamin D, the idea sprang up that ergosterol (or its esters) is the only activatable sterol. The recently isolated lumisterol and tachysterol need not be cited as exceptions to this assumption, for they are merely way-stations between ergosterol and calciferol, and apparently they do not occur in nature.

Nevertheless, even with this allowance, ergosterol cannot be said to be the only provitamin, although it is practically a very important one. A method used by the English and German investigators in 1926 to destroy provitamin in cholesterol was treatment with charcoal in boiling alcohol. Bills (1925) had used the same treatment, but found the cholesterol activatable. Jendrassik and Keményffi (1927) reported that cholesterol treated with charcoal was not activatable, but that cholesterol purified by bromine always retained a fraction of its activatability. To explain the residual activatability in the latter case,

they postulated the existence, under suitable conditions, of an equilibrium between cholesterol and provitamin.

A reinvestigation by Bills, Honeywell and MacNair (1928) showed that prolonged treatment of cholesterol with charcoal, or repeated treatment with bromine, always left cholesterol with a modicum of activatability. The cholesterol thus purified showed very little absorption, but in high concentrations it exhibited a series of absorption bands with maxima at 315, 304, 293.5, 282, and 269 mμ. General absorption obscured the region at 260 mμ and below. The bands at 293.5, 282, and 269 mμ could have been due to ergosterol, but this seemed improbable, since more than 99 per cent of the original "ergosterol" was shown to have been destroyed after one treatment with bromine, and the intensity of the bands was not further reduced by two additional treatments. Although the shadow of a doubt persisted that ergosterol was concerned, the authors concluded that the activatability was due either to cholesterol itself or to an unknown impurity.

Kon, Daniels and Steenbock (1928) confirmed the observation that cholesterol, purified by charcoal or bromine, may still exhibit slight activatability. They found, however, that treatment with potassium permanganate gave a cholesterol devoid of activatability. In spite of the known sensitiveness of ergosterol to bromine, they concluded that ergosterol was the source of the activatability. The reviewer interprets their work as indicating that sufficiently pure cholesterol is not activatable, but not as demonstrating the nature of the activatable impurity in other cases.

Koch, Koch and Ragins (1929) again found that cholesterol purified by bromine was activatable. So also was a specimen treated with permanganate. They made the noteworthy observation that the slight activatability of the treated cholesterol preparations was increased 25-fold by heating the sterol after purification. Koch, Koch and Lemon (1929) found that the heated specimens showed strong general absorption. It must be that the provitamin in the heated sterols was mainly, if not entirely, a transformation product of cholesterol itself, and not any contaminant in the original crude cholesterol which escaped destruction.

Schoenheimer (1931) applied to Koch's experiments the interpretation that cholesterol, when heated, undergoes a simultaneous hydrogenation-dehydrogenation reaction, resulting in the formation of a completely saturated sterol and a highly unsaturated, ergosterol-like body which is activatable.

Lastly, the analytical determinations of Windaus, v. Werder, and

Gschaider (1932) and Windaus and Lüttringhaus (1932) have established with fair certainty that cholesterol has one fewer carbon atoms than ergosterol. The provitamin in Koch's preparations therefore could not be ergosterol, although it might possibly be a lower homolog.

It is thus easier now than heretofore (*cf.* Loofbourow, 1931) to conceive that the provitamin in cholesterol subjected to ergosterol-destroying treatment is not ergosterol. Apparently in the act of regenerating cholesterol from its dibromide, or of treating it with mild oxidizing agents such as charcoal and permanganate, and certainly in the process of heating it, one produces a provitamin of fewer carbon atoms than ergosterol. The occasional success in the production of non-activatable cholesterol (Jendrassik, Steenbock), rather than the usual failure, is the phenomenon more in need of an explanation.

The consideration of the number of carbon atoms in the provitamin makes it clear that the vitamin produced by irradiating ergosterol-free cholesterol is neither identical nor isomeric with calciferol. Thus in two instances, the first being the fuller's earth product, antiricketic sterol derivatives other than calciferol have been shown to exist. Since both of these examples have only theoretical significance, it is time to turn to instances of practical importance.

In 1928 a solution of ergosterol, irradiated in this laboratory and standardized on rats, was administered to chickens by Dr. C. W. Carrick of Purdue University. Carrick found that as a preventive of rickets it compared very poorly with a rat-equivalent amount of cod liver oil (private communication). This led to a systematic investigation of the action of activated ergosterol in the chicken, the results of which were published by Massengale and Nussmeier (1930). Under the conditions of their experiment the vitamin D of cod liver oil appeared to be roughly 100 times as effective as that of irradiated ergosterol, the two preparations being standardized with rats. A brief note by Hess and Supplee (1930), and papers by Mussehl and Ackerson (1930), Supplee, Flanigan, Kahlenberg and Hess (1931), Russell and Klein (1931), Tully, Hauge, Carrick and Roberts (1931), Hunter, Dutcher and Knandel (1932), Steenbock, Kletzien and Halpin (1932), Branion and Smith (1932), King, Hull and Hall (1933), DeVaney, Munsell and Titus (1933), and Bethke, Record and Kennard (1933), amply confirmed the observation that the vitamin D of irradiated ergosterol is far less effective for the chicken than the rat-equivalent amount of vitamin D from cod liver oil. Or, to express it with equal accuracy, irradiated ergosterol is far *more* effective for the rat than the chicken-equivalent amount of cod liver oil.

From the investigations just mentioned, and particularly from unpublished studies made in this laboratory, it appears that the observed differences between the two antiricketic agents are not due to the presence or absence of vitamin A, nor to the nature of the oily vehicle. It is thus evident that the vitamin D of irradiated ergosterol is a different substance from that contained in cod liver oil.

The human being comes between the chicken and the rat, but nearer the rat, in response to the two forms of vitamin D. It is noteworthy that, in spite of the almost immediate adoption of irradiated ergosterol as a therapeutic agent, nearly three years passed before evidence was obtained that a rat unit of this form of vitamin D exerts on the infant a quantitatively different effect than a rat unit of cod liver oil. While this is no compliment to the preciseness of clinical medicine, it is doubtless explained in part by the fact that precise methods of standardization with rats were not in general use for either cod liver oil or activated ergosterol.

Evidence that cod liver oil and irradiated ergosterol (Viosterol) in amounts equipotent for rats do not exert the same degree of antiricketic effect on children, came largely from the studies of Hess, Lewis, and Rivkin (1930) and Hess and Lewis (1932, 1933). These workers found that about 3 rat units of the ergosterol preparation were clinically equivalent to 1 rat unit of cod liver oil. The satisfactory experience of physicians with activated ergosterol, in the face of this anomaly, was attributed to the fact that the customary dose of Viosterol is ample to counterbalance the discrepancy in rat-unit effectiveness.[2]

Several types of vitamin D milk have been used in the treatment of rickets. One is prepared by feeding irradiated yeast to the cow; another by directly irradiating liquid or dried milk. Hess and Lewis (1933) found that twice as many rat units of the first type are required to heal rickets in babies as are required of the second type. If this observation is confirmed, it will be of particular significance in showing that two forms of vitamin D are produced in the irradiation of provitamin-bearing substances. Krauss, Bethke, and Monroe (1932) found that butterfat from the "yeast milk" was less than one-fourth as effective as the rat-equivalent amount of cod liver oil for preventing rickets in chickens.

The experiments of Hess and Lewis (1933) and Mitchell, Eiman, Whipple, and Stokes (1932) indicated that the efficiency of vitamin D

[2] Not all students of vitamin D therapy accept these findings of Hess. Shelling is one who considers activated ergosterol and cod liver oil equally effective for the infant.

milks in human rickets is high in comparison with cod liver oil or Viosterol. Unlike the comparison of one milk against another, this does not necessarily point to the existence of a multiplicity of forms of vitamin D, inasmuch as the observed differences in effect may be due at least partly to differences in dispersion and assimilability. The phenomenon nevertheless is one of considerable practical significance and great theoretical interest.

Very recently a paper appeared in which chemical differences between different forms of vitamin D seem to have been demonstrated. Ender (1933), taking advantage of the extraordinary richness of tunafish liver oil, prepared a concentrate which had about 100,000 times the antiricketic potency of cod liver oil. (Calciferol, in comparison, is about 400,000 times as potent as average cod liver oil.) Ender's preparation was therefore sufficiently pure to justify a comparison of properties. He found that it exhibited only slight optical activity, in contrast with the strong dextrorotation of calciferol. This could be due, of course, to the presence of a levorotatory impurity. The absorption curves were more significant, for they showed relatively weak general absorption in the region of the absorption maximum of calciferol. The tuna oil vitamin, like calciferol, is an alcohol, for it formed an ester with phthalic anhydride. Its rate of reaction with phthalic anhydride was, however, much greater than that of calciferol.

Bills suggested (1927b) in the conclusion of a study on the synthesis of vitamin D by fish, that "quite possibly vitamin D is not a single substance, but a mixture or series of substances as variable as the sterols with which it seems to be almost inseparably associated." It now appears, from the studies reviewed above, that the existence of at least three forms of vitamin D is established.

*Origin of vitamin D in fish oils.* In the discussion of distribution it was shown that vitamin D rarely, if ever, occurs in living plants. In the animal kingdom it is widespread, but only in the fishes abundant. Its origin and significance in fishes is obscure. An explanation was offered by Steenbock and Black (1925), in a revival of the old suggestion that it is formed in the insolation of plankton which in turn is ingested by little fish, and these by larger fish, etc. Against this hypothesis is the observation by Leigh-Clare (1927) that the marine diatom, *Nitzschia closterium*, which synthesizes "vitamin A" (carotene ?), fails to effect any genesis of vitamin D, even when grown under conditions of maximum insolation. Drummond and Gunther (1930) obtained essentially negative findings with both phytoplankton and zooplankton collected

from the ocean. Belloc, Fabre, and Simonnet (1930) found that zooplankton collected in spring contained "ergosterol" but no vitamin D, while zooplankton taken in midsummer showed "ergosterol" with perhaps vitamin D. Their evidence, although not convincing, may have some significance in view of the July change in vertical distribution of zooplankton (Russell, 1930). It would seem that if fish obtain vitamin D from plankton, they must salvage it with extraordinary economy.

Bills (1927b) observed that the capelin, *Mallotus villosus*, which is the principal food of the Newfoundland codfish, is a poor source of vitamin D. He also noted that marine mammals—the seal and whale, which subsist on fish, have little or no vitamin D in their blubber fat. In piscicultural experiments with young catfish, *Ictalurus punctatus*, he obtained evidence, inconclusive to be sure, "that the substance, or group of substances, known as vitamin D, may be synthesized by fish." The enormous differences in potency of the liver oils of different species of fish are revealed in table 2. For example, tuna liver oil contains 100 to 700 times as much vitamin D as cod liver oil, and infinitely more than sturgeon liver oil. Such differences in potency are accounted for more reasonably on the basis of species differences in the ability to synthesize, rather than to salvage and store.

Several investigators have associated a low vitamin D content in the liver oil with imperfect bone formation in the so-called cartilaginous fishes—elasmobranchs and cyclostomes—including representative sharks, dogfish, skates, rays, chimeras, and lampreys (Poulson, 1929; Schmidt-Nielsen and Schmidt-Nielsen, 1930; Callow and Fischmann, 1931; André and Lecoq, 1932). Examples of this most interesting relationship are obvious in table 2. It is evident, however, from table 2, that *some* cartilaginous fishes have more vitamin D than *some* fishes with hard bones; also that sturgeons, which are usually classified as true fishes in spite of their skeletal weakness, have the lowest vitamin D content of all.

Irradiation of the body surface appears to play no part in the formation of vitamin D in fish. Bills (1927b) observed that catfish are very sensitive to ultraviolet irradiation, yet do not elaborate more vitamin D as a result of it. Schmidt-Nielsen and Schmidt-Nielsen (1930) found that the basking shark, *Cetorhinus maximus*, one of the elasmobranchs, has comparatively little vitamin D in its liver oil, in spite of the fact that it basks for hours at the surface of the water, and feeds on plankton.

*Origin of vitamin D in the higher animals.* The inability of higher animals to synthesize vitamin D is shown by the incidence of rickets.

Except for occasional amounts gained by eating such foods as fish and eggs, the vitamin must normally be obtained by exposure of the body surface to the sun. Reference has already been made to the healing of rickets by sunshine, the activatability of wool fat, and the occurrence of provitamin in the skin. The mechanism of vitamin D formation is thus fairly obvious, but special studies have made it even more clear.

Hess and Weinstock (1925a) demonstrated that irradiated skin is antiricketic, and that irradiated sterol exerts its action when administered subcutaneously. Rekling (1927) found that irradiation did not protect rats from rickets when they were prevented from licking their fur. Hou and Tso (1930) found that the skin of normal rabbits was slightly antiricketic, the dorsal skin more so than the ventral, but that the skin of rickety rabbits or of normal rabbits reared indoors was without protective action. Hou (1930b) noted that the effectiveness of ultraviolet irradiation for curing rickets in rabbits was almost lost when the skin had been previously washed with ether. Rowan (1928) observed that birds of prey on a meat diet developed rickets, and that the addition of feathers to the diet supplied protection. He suggested that the preen gland is concerned with the formation of vitamin D. Hou (1928, 1929, 1930a, 1931) made an elaborate study of the formation of vitamin D in birds. In brief, his findings were as follows. Birds differ from mammals in having only one gland of a sebaceous nature. This is the glandula uropygialis, or preen gland. Preen gland oil contains "ergosterol," which birds, by preening, distribute over their feathers and effectively expose to sunlight. The vitamin D is either ingested by swallowing the feathers, or absorbed by the skin from the feathers. The feathers and skin of normal birds were shown to be antiricketic, but in rickety birds, or birds whose preen glands had been removed, the feathers and skin had little, if any, antiricketic action. Removal of the preen gland made the birds susceptible to rickets, and rickety birds without the gland were not benefited by exposure to ultraviolet radiation or sunshine.

Thus Hou (1929) was led to see that ". . . . vitamin D or its precursor is principally derived from the oil secretion rather than from the diet. This may be more or less true for all birds. For although nocturnal birds, and the carnivorous animals which prey upon other forms of 'feather and fur,' may derive their vitamin D supply mainly from their victims, yet the source of the vitamin in the prey would appear to lie in the oil secretion. This may explain the absence of oil gland in some species of birds . . . . and the necessity of adding rabbits or small birds

with the fur or feather intact, to the diet of young carnivora in captivity to ensure their successful development. . . . . Further, it is commonly known that if herbivora, e.g., horses, are scrubbed thoroughly with soap and water, they do not thrive. It may thus be inferred that the sebaceous secretion, which is the homologue of the secretion of the preen gland of birds, . . . . is an important source of vitamin D in the mammal."

Hou (1931) found that rickety chickens with preen glands removed could be cured by irradiating the feet, even though irradiation of the body or of the head was ineffective. In this case the substance activated was obviously neither preen gland oil nor circulating blood, but something in the tissues of the feet which was directly absorbed.

Evidence enough has been presented to show that the higher animals obtain vitamin D in at least three ways, the relative importance of which must vary with habits, requirements, and opportunities: *a*. By eating such foods as eggs, fish, whole furred or feathered animals, and insolated dead vegetable tissues; *b*, by ingesting insolated sebaceous matter in the process of neatening the body—licking and preening; and *c*, by directly absorbing the products of insolation formed on or in the skin.

THE PHYSIOLOGY OF VITAMIN D. Except for the observations that rickets is healed by calciferol and that toxic effects result from too much of it, few physiological studies have been reported on any pure form of vitamin D. One must await the isolation of the several forms of the vitamin before enduring knowledge of their physiology and toxicology shall be available. As a guide to the future, however, a mere outline of past studies on crude vitamin D-bearing mixtures is worth while. From this viewpoint studies with fish oils or with irradiated ergosterol preparations other than the toxic Vigantol can be considered. Most investigations have been carried out with cod liver oil, or with the commercial solutions of irradiated ergosterol known as Radiostol, Viosterol, etc.[3] For details on the physiological action and therapeutic use of vitamin D the reader must examine the more specialized reviews by Goldblatt (1931), the Medical Research Council (1932), Park and Eliot

[3] There are, in the various countries, about 20 commercial irradiated ergosterol solutions, each sold under a trade name. In an attempt to simplify nomenclature, the publications of the American Medical Association refer to all such products as Viosterol, the trade name generally used in the United States. In several instances this editorial policy has led to confusion, particularly where products containing toxisterol were discussed. There is no reason to consider even the several makes of Viosterol identical.

(1933), Laurens (1933), and others. Goldblatt's is the most extensive monograph on experimental rickets, containing, in fact, 2723 references. The Medical Research Council's report is the most authoritative brief survey of the vitamins. Park and Eliot supply the outstanding chapter on vitamin D from the therapeutic standpoint. Laurens gives a detailed résumé of studies on vitamin D, with emphasis on the rôle of radiant energy.

*Systems of units and methods of assay.* Medicinal cod liver oil, either of Norwegian or Newfoundland origin, contains about 100 I.U. of vitamin D per gram, and only rarely deviates to half or twice this figure (Bills, Honeywell, Wirick and Nussmeier, 1931; Nelson and Walker, 1932; Coward, Dyer and Morgan, 1932). This is true, in spite of the fact that the oil from livers of individual codfish may vary several hundred times in potency (Hess, Bills and Honeywell, 1929). It is therefore possible to gain a general idea of the unitage provided by the doses of cod liver oil which experimenters in various countries have administered, even when the doses were not measured in biological units.

On the other hand, unit comparison of the doses of irradiated ergosterol which various workers have administered is frequently difficult and sometimes impossible. From the beginning, the preparations of irradiated ergosterol available in different countries have differed not only in the potency of the irradiation-product which they contained, but in the concentration thereof per cubic centimeter of oily vehicle. Statements of doses measured in milligrams, drops, or cubic centimeters are therefore almost meaningless unless the biological unitage per measure is defined. Equally obscure are statements of doses in terms of "units" or "rat units," unless the particular system of units used is specified. Bills, Honeywell, Wirick, and Nussmeier (1931) published a table comparing the several systems of units in common use for the assay of fish oils, activated ergosterol, and other vitamin D products. Extended, and corrected after hundreds of calculations and assays, it is reproduced below as table 3.

The experimental 100 D solutions, standardized in this laboratory, and used in the early work of some authors, notably Alfred Hess and Julius Hess, were 100 times as potent as average cod liver oil. On the other hand, the commercial solutions of "100 D" or "250 D" strength have had 100 or 250 times the potency of the so-called Wisconsin or A.M.A. standard, which is 2.8 times weaker than ordinary cod liver oil. It is expected that the adoption of international units for the 1934 in-

terim revision of the U.S. Pharmacopeia will be followed by a discontinuance of the present "D" nomenclature, and of the divers systems of units which have been the source of confusion in the past. In the following pages, doses are expressed in international units (I.U.), when sufficient information is available to justify the translation.

TABLE 3

*Expressing the vitamin D content of average cod liver oil in terms of various systems of units*

| | |
|---|---|
| International, or League of Nations (1931) system | 100 units per gm. |
| United States Pharmacopœia (1934) system; same as International | 100 units per gm. |
| British, or Medical Research Council (1930) system* | 100 units per cc. |
| Steenbock system (Steenbock, Hart, Hanning, and Humphrey, 1930)** | 37 units per gm. |
| American Drug Manufacturers' Association, or Holmes (1931) system** | 350 units per gm. |
| Oslo (old), or Poulsson and Lövenskiold (1928) system† | 110 units per gm. |
| Oslo (new), or Poulsson and Ender (1933) system‡ | 160 units per gm. |
| German system (Scheunert and Schieblich, 1929; Holtz and Schreiber, 1930; Holtz, Laquer, Kreitmair, and Moll, 1931): | |
| Rat units* | 15 units per cc. |
| Clinical units (1 clinical unit = 100 rat units)* | 0.15 units per cc. |
| American Medical Association (1931) system§ | 2.8 "D" potency |

* Obsolete; replaced by international units.

** Obsolescent; to be replaced by international units.

† Obsolete; vaguely defined.

‡ Used in Norway for liver oils; vaguely defined; may soon be replaced by international units.

§ In the earlier descriptions of Steenbock's hypothetical 1 D oil (American Medical Association, 1929, 1930) a typographical error made it appear that 1 Steenbock unit was contained in 0.75 mgm. of oil; the 1931 and subsequent descriptions specify 75 mgm. Either value is misleading. Abandoned, 1934.

The development of precision methods of assay, independent of the system of units employed, has been carried out by several American and English groups. Procedures in which the probable error has been studied include those of Bills, Honeywell, Wirick and Nussmeier (1931), Bourdillon, Bruce, Fischmann and Webster (1931), Dyer (1931), Bourdillon and Bruce (1932), Morgan (1932), Coward and Key (1933), Bills and Imboden (in preparation), and others.

*Toxicity.* It is characteristic of vitamin D that the range between its

ordinary therapeutic dose and the dose which produces adverse symptoms is extraordinarily wide; wider, perhaps, than is the case with any other potent drug. This is fortunate, for probably no potent substance enters the bodies of animals in more variable amounts than vitamin D. Animals depend for their supply mainly upon the action of sunshine on the sterol of the skin, and obviously the amounts obtained in this way vary enormously with the weather and the seasons.

Bills and Wirick (1930) summarized the knowledge on the toxic limit as follows. "In the earliest reference to the action of large doses of irradiated ergosterol, Rosenheim and Webster (1927c) reported that 10,000 times the minimum antiricketic dose was not lethal to rats. Kreitmair and Moll (1928), using 'enormous doses,' described pathological findings for several species, and observed a species difference in susceptibility. Collazo, Rubino and Varela (1929) observed that rats died within 6 weeks when fed the McCollum high calcium rickets-producing diet 3143 plus 50,000 times overdosage of activated ergosterol. The important work of Harris and Moore (1928) demonstrated that 1000 times overdosage of vitamin D was harmless to rats, whereas 100,000 times overdosage was rapidly fatal. The agent which produced the characteristic lesions was apparently the activated ergosterol *per se*, and not unchanged ergosterol or decomposition products. Light, Miller, and Frey (1929) reported that while 100,000 times overdosage was fatal, 10,000 times had no effect on growth during 6 months. Numerous other papers, mostly quite uncritical, have dealt with the effects of extraordinary doses. In nearly every case the authors failed to determine the potency of the product which they employed. They stated only the quantity of irradiated ergosterol in milligrams."

Bills and Wirick (1930), in a study involving large numbers of rats from infancy to late maturity, found that 1000 times overdosage was just perceptibly harmful, 4000 times overdosage definitely injurious, and 40,000 times overdosage strongly toxic to rats receiving a normal diet.

The composition of the diet exerts a major influence on the effects produced by vitamin D. Calcium salts potentiate the vitamin. Concerning their influence, and that of phosphates, there is an extensive literature, of which the following references are representative: Bills and Wirick (1930), Harris (1930), Hoyle (1930a, b), Duguid, Duggan, and Gough (1930), Harris and Innes (1931), Jones and Rapoport (1931), Shelling (1932b), Shelling and Asher (1932), Hauch (1933), and Gough, Duguid and Davies (1933).

The margin of tolerance for man has not been determined as accurately as for the rat; it is wide, but apparently not as great as in the rat. The consensus of opinion of leading investigators is that the smallest dose of vitamin D which can be relied upon to prevent rickets or to heal mild rickets in infants is about 3000 I.U. per day in the form of irradiated ergosterol, or 1000 I.U. per day in the form of cod liver oil, the dosage in either case being continued over several weeks. This may be regarded as the ordinary therapeutic dose, and so, after painstaking translation into I.U. of the excessive doses which various workers have administered, one can arrive at an estimate of the overdosage which is tolerated by the human being.

Hess, Poncher, Dale and Klein (1930) administered as much as 40 times the ordinary dose of irradiated ergosterol to babies for 6 months, and noted no untoward effect other than a tendency to constipation and colic. A greatly increased appetite resulted, but the gain in weight was not commensurate with the food intake. Reed and Seed (1933) administered 300 times overdosage for several days to patients with parathyroid tetany. The tetany was alleviated, and no toxic conditions developed. Rappaport and Reed (1933) have shown, however, that doses of this magnitude, given to adults with intact parathyroids, are sufficient to initiate mild toxic effects in some individuals when continued for several weeks. This agrees fairly well with the findings of Crimm (1932) and Crimm, Strayer, Watson and Heimann (1933), who worked with normal and tuberculous individuals receiving a diet fairly rich in calcium. They found that 150 times overdosage, continued for several days, resulted merely in a hypercalcemia which was compatible with well-being. Prolongation of the treatment occasioned some toxication in certain instances.

The production of toxic effects depends upon *a*, the daily dosage; *b*, the duration of dosage; *c*, the route of administration; *d*, the calcium and phosphorus content of the diet; *e*, the age and activity of the subject; *f*, the individual susceptibility; *g*, the species susceptibility; and other factors. There is also the special factor of kidney function, which becomes particularly important in renal rickets, a disease for which vitamin D in normal doses is ineffective, and in larger amounts contraindicated (Shelling and Hopper, 1934). With so many variables involved, it is impossible to state a toxic limit true of all cases. But analysis of the work of Crimm and of Reed indicates that in man toxic effects begin to appear when approximately 600,000 I.U., or 200 times the ordinary dose, of properly irradiated ergosterol is administered daily over several weeks.

Thus it seems that the margin between the ordinary and the toxic doses is less for man than for the rat. However, on the basis of the dose per kilogram of body weight required to produce either antiricketic or toxic effects, man is distinctly less sensitive than the rat to vitamin D. Other species show noteworthy differences in susceptibility to large doses. Kreitmair and Moll (1928) compared the susceptibility of rats, mice, guinea pigs, rabbits, cats, dogs, chickens, and axolotls. Cats and rabbits were the most sensitive; chickens and axolotls were extremely resistant. Taylor, Weld, Branion, and Kay (1931) compared rats, mice, chickens, guinea pigs, rabbits, cats, and dogs. The order of listing was roughly the order of increasing susceptibility. Taylor, Weld, Branion, and Kay, and also Harris and Moore (1929) found young animals were more susceptible than old, but Hauch (1933) reported the old to be the more susceptible.

The lethal dose of vitamin D for man is unknown. Almost the largest doses ever given were those administered by Rappaport and Reed (1933) and Reed (1934) to patients with hay fever and other ailments. At the maximum, the doses amounted to 3 cc. daily of a concentrated irradiated ergosterol solution which had been standardized in this laboratory, and was known to contain 1,000,000 I.U. of vitamin D per gram of oil. In other words, this solution was 10,000 X cod liver oil in vitamin D potency. Allowing for the specific gravity, 0.92, one calculates that 3 cc. of solution contained 2,760,000 I.U. of vitamin D (the equivalent, in rat units, of 30 liters of cod liver oil). Such an enormous daily dose, 920 times the ordinary therapeutic dose of irradiated ergosterol, was more than some individuals could tolerate with comfort, yet it produced no serious poisoning. The largest doses ever administered were those given by Spies and Hanzal (1934) to patients who were about to die of disease. The maximum dosage, over 18,000,000 I.U. per day,[4] or more than 6000 times the ordinary amount, produced hypercalcemia, but no pathological calcification of tissues.

In view of these findings, an interpretation of the so-called Vigantol poisoning is in order. Around 1929 there appeared in the clinical literature numerous accounts of severe and sometimes fatal poisoning following the administration of a proprietary "vitamin D" solution known as Vigantol (Bamberger and Spranger, 1928; Wiskott, 1928; Adam, 1928; Degkwitz, 1928; Bamberger, 1929; György, 1929; Put-

[4] The solution used was assayed in this laboratory. It contained 1,000,000 I. U. of vitamin D per gram. Unfortunately, the authors described it in the ambiguous "D" terminology.

schar, 1929; Klausner-Cronheim, 1930; and others). Hess and Lewis (1928a, b) also reported, from New York, adverse symptoms from the use of the imported Vigantol. Vigantol at this time was an oily solution containing in each cubic centimeter 10 mgm. of an ergosterol irradiation-product which was presumed to be vitamin D. Doses were not measured in biological units, but in milligrams of the irradiation-product. About 1 mgm. was required to heal rickets; doses of from 20 to 5 mgm., sometimes even less, produced in children a train of symptoms, of which kidney involvement was the most alarming.

The dosage in milligrams could hardly supply enough vitamin D units to account for the observed effects, for the potency of pure calciferol is only 40,000 I.U. per mgm. Toward the explanation of Vigantol toxication Göttche and Kellner (1931) established the important point that the toxic and antiricketic doses of different irradiated ergosterol solutions did not run parallel, and that with Vigantol, in particular, the margin of safety, or "therapeutic index," was anomalously narrow. Like previous investigators, they suggested, but were unable to prove, that a technical fault in manufacture was responsible. In the light of present knowledge, the whole mystery of Vigantol poisoning becomes clear when one assumes that this product was irradiated in accordance with the patent issued to its manufacturer (I.G. Farbenindustrie Aktiengesellschaft, 1928). The specifications of this patent are precisely directions for producing, under spectrographic regulation, *not vitamin D, but toxisterol, i.e., substance 248, in maximum concentration.*[5]

Partly as a result of the Vigantol difficulty, knowledge of the action of vitamin D in large doses is in confusion. Many (but one cannot always be certain which) of the early German studies on hypervitaminosis D with animals may be in reality studies on the action of substance 248. Toxisterol, or substance 248, is readily detected spectrographically, and easily avoided in the practice of activation. However, even those preparations of irradiated ergosterol in which it is not a factor are capable of producing hypervitaminosis D, for the one form of vitamin D which has been isolated in the crystalline state, namely, calciferol, is toxic in sufficiently large doses (Askew, Bourdillon, Bruce, Callow, Philpot and Webster, 1932; Dale, Marble and Marks, 1932; Gough, Duguid and Davies, 1933; Hesse, 1934). Whether the toxic effects of calciferol and toxisterol differ in kind, as well as in degree, is uncertain, and whether calciferol is less, or more toxic than the other forms of

[5] It is understood that the composition of Vigantol has lately been changed, so that toxisterol is no longer a factor therein.

vitamin D is unknown. In passing, it is to be noted that derivatives of the ergosterol irradiation-products, produced by heat, reduction, or oxidation, have toxic calcifying properties, and form the subject of numerous recent contributions (Windaus, 1930; Holtz and Schreiber, 1930; Windaus and Auhagen, 1931a, b; Windaus, 1931a; György and Popoviciu, 1931; Popoviciu, 1931; Windaus, Busse, and Weidlich, 1931; v. Brand, Holtz and Putschar, 1932; Popoviciu and Nitzescu, 1932; Windaus, v. Werder and Lüttringhaus, 1932; Holtz, Gürsching and Kraut, 1933; Holtz, 1933; Snapper, 1934; and others).

Although fatal vitamin D poisoning has not been observed in man, it is theoretically possible, and so, for the benefit of experimentalists who may have occasion to make physiological studies on patients receiving massive doses, the following warning symptoms of toxic action are mentioned: A sense of well-being and increased appetite changing to nausea and loss of appetite; vomiting, cramps, diarrhea, and frequent urination; sometimes neuralgia along the course of the mandibular branch of the trigeminal nerve, tenderness of gums and teeth, pain in muscles and joints; dizziness, muscular weakness, headache, haziness of memory, and occasionally numbness and tingling in the extremities (Crimm, 1932; Rappaport and Reed, 1933; Reed, 1934). These symptoms are usually, but not invariably, associated with a hypercalcemia in excess of 15 or 16 mgm. per cent. Discontinuance of the drug, and intravenous administration of saline solution bring prompt recovery (Crimm, Strayer, Watson and Heimann, 1933; Crimm and Strayer, 1934b).

The early observations on the lesions produced in animals by enormous overdoses of irradiated ergosterol have been reviewed by Duguid (1930b). Many painstaking histological studies, especially in the German literature, are clouded by the uncertainty as to whether the lesions described were due to vitamin D or to toxisterol (*Vigantolschaden*). Metastatic calcifications are the most obvious effect, but not the only one (Harris and Innes, 1931; Harris, 1932; Shelling and Asher, 1932; Gough, Duguid and Davies, 1933). Animals show large deposits of calcium in the arteries, myocardium, lungs, kidneys, stomach, and intercostal muscles. Urinary calculi are sometimes seen, and also a parenchymatous degeneration in the kidney. The calcium content of any tissue may be significantly increased (Reed, Dillman, Thacker and Klein, 1933). The calcium generally comes from the diet, but if this be deficient, or the overdosage tremendous, it may be withdrawn from the skeleton (Harris and Innes, 1931; Shelling and Asher, 1932; and many others).

The vascular calcifications are confined more to the media, as in the Mönckeberg type of sclerosis, than to the intima, as in ordinary arteriosclerosis (Duguid, 1930a; McGowan, Cunningham and Auchinachie, 1931; Shelling and Asher, 1932; Ham and Lewis, 1934). A similar picture is seen with toxisterol, *i.e.*, Vigantol (Schmidtmann, 1928; Herzenberg, 1929; Schiff, 1930; Laas, 1930). According to Shelling and Asher, an erroneous impression that vitamin D plays a rôle in the etiology of human arteriosclerosis may come from a confusion of the two forms of calcification. Schönheimer (1932), in an analysis of the sterols extracted from 120 sclerotic human aortas, found no vitamin D. This would suggest that the vitamin is not a usual factor in the development of arteriosclerosis. Reed (1934) observed that huge doses of irradiated ergosterol, when administered for long periods to elderly patients, produced no hypertension.

Formerly it was held that the lesions, as seen in the arteries of animals, represent primarily a degenerative change in the muscular layer, followed by calcification. It now appears that in some instances, at least, the calcification is primary and the degeneration secondary (Ham, 1932; Reed, Dillman, Thacker and Klein, 1933). There may be special significance in the fact that these workers used an irradiated ergosterol solution which is toxisterol-free. It is not to be doubted that calcification readily occurs in previously damaged areas, *e.g.*, the tuberculous lesions of Spies (1931), but the damage which sometimes appears to precede deposition in hypervitaminosis D does not seem to be especially fundamental, for animals may recover rapidly after severe poisoning (Harris and Moore, 1929; Bills and Wirick, 1930; Watchorn, 1930; Harris and Innes, 1931; Spies and Lyman, 1932; and Reed, 1934). The general subject of pathological calcification is reviewed by Barr (1932).

*Absorption, transport, and elimination.* Vitamin D can enter the circulation by any of several routes. Antiricketic agents administered by mouth are absorbed presumably through the intestinal wall. Absorption through the skin has already been mentioned. In this connection one recalls the time-honored practice of giving cod liver oil by inunction, and the experimental work of Hume, Lucas and Smith (1927) and Taylor, Weld, Branion and Kay (1931), which showed that irradiated sterol is absorbed through unbroken skin. Injections also are effective, whether intravenous (Reed and Thacker, 1931; Taylor, Weld, Branion and Kay, 1931; Dale, Marble and Marks, 1932), intraperitoneal (Soames, 1924; Stepp, 1925; Lévy-Solal, Christou and Dalsace, 1926; Koehne and Mendel, 1929; Reed and Thacker, 1931; Spies and Lyman,

1932; Russell, Taylor and Wilcox, 1932), intramuscular (Wilkins and Kramer, 1927; Koehne and Mendel, 1929), or subcutaneous (Hess and Weinstock, 1925a; Lévy-Solal, Christou and Dalsace, 1926; Kramer, Kramer, Shelling and Shear, 1927; Tammann, 1928; Koehne and Mendel, 1929; v. Brand and Holtz, 1929, 1931; Taylor, Weld, Branion and Kay, 1931; Greaves and Schmidt, 1932, 1933, 1934a, b; Daly, 1933).

It would seem that vitamin D passes the placental wall only with difficulty. At least it is true that animals *in utero* cannot store enough vitamin D to afford them more than temporary and partial protection against rickets after birth, even when their mothers are liberally provided with the vitamin (Hess and Weinstock, 1924a, b; Bills, 1927b; and especially Bills and Wirick, 1930). In theory, the immature cartilage cells of the fetus could not utilize vitamin D, even if its calcifying action were available to them. Thus it appears that mammals do not obtain much of the skeleton-hardening factor until the period of suckling, whereas birds, which are hatched with skeletons more developed than those of many mammals, have available in embryonic life a supply of vitamin D from the egg yolk.

Although milk is not a rich source of vitamin D, there is ample demonstration that vitamin D is transferred to milk a little more readily than to the fetus. It has been shown with several species that the vitamin D which is produced by insolating or irradiating the mother can be detected in the milk (Steenbock, Hart, Hoppert and Black, 1925; Gowen, Murray, Gooch and Ames, 1926; Chick and Roscoe, 1926; Falkenheim, Völtz and Kirsch, 1926; Hess, Weinstock and Sherman, 1926, 1927; Bruck-Biesok, Pirquet and Wagner, 1927; Mitchell, Eiman, Whipple and Stokes, 1932; and others). Likewise, vitamin D which is taken by mouth appears in the milk, if the amount ingested is sufficiently large (McCollum, Simmonds, Becker and Shipley, 1927; Wachtel, 1929; Bills and Wirick, 1930; Hart, Steenbock, Kline and Humphrey, 1930; Scheer and Sandels, 1930; Hess, Lewis, MacLeod and Thomas, 1931; Wyman and Butler, 1932; Gerstenberger and Horesh, 1932; Bunker, Harris and Eustis, 1933; and others). Usually the administration of comparatively weak antiricketic agents, such as cod liver oil, has occasioned little, if any, enhancement of the antiricketic potency of the milk. The more potent sources of vitamin D, such as the irradiated yeast which is fed to dairy cattle, can cause a distinct increase in the vitamin D content of the milk. However, a relatively large amount of vitamin D must be given, in order to effect a small enrichment of milk (Krauss and Bethke, 1931; Thomas and MacLeod, 1931; Hess, Light, Frey and

Gross, 1932; Krauss, Bethke and Monroe, 1932; Krauss, Bethke and Wilder, 1933).

Eggs normally are a fair source of vitamin D, and the vitamin is transferred to them more readily than to milk. Nevertheless, the transfer is not remarkably efficient. It is well known that when good sunlight is not available, either ultraviolet radiation or fish oil must be supplied to hens in order to maintain the production and hatchability of their eggs. Hughes, Payne, Titus and Moore (1925) observed that the amount of ultraviolet radiation received by hens is an important determinant of the vitamin D potency of their eggs. Hart, Steenbock, Lepkovsky, Kletzien, Halpin and Johnson (1925) found that the egg yolk from irradiated hens was 10 times as antiricketic as that from non-irradiated hens. The vitamin D of cod liver oil also finds its way to the yolk of eggs (Bethke, Kennard and Sassaman, 1927). In quantitative experiments at this laboratory egg yolk oil from pullets receiving 2 per cent of cod liver oil in their ration was found to be 0.7 as potent in vitamin D as the cod liver oil. The substitution of an irradiated ergosterol solution containing 10,000 times as many rat units as the cod liver oil resulted in an increase of only 185 times in the vitamin D potency of the yolk oil (McDonald and Massengale, 1932). DeVaney, Munsell and Titus (1933) found that the vitamin D of irradiated ergosterol is transferred to eggs less efficiently than the vitamin D of cod liver oil, but in proportion to the amount administered.

Vitamin D was detected in the circulating fluid by Hess, Light, Frey and Gross (1932). The blood of cows which had eaten irradiated yeast was definitely antiricketic, the plasma more so than the corpuscles. The liver of one of these cows was tested and found to contain the vitamin, but not in much greater concentration than the blood. The blood of infants receiving antiricketic medication has also been shown to contain vitamin D (Hess, Weinstock and Gross, 1933). In the experiments of Coppens and Metz (1933a), vitamin D was found in the blood of dogs which had been given irradiated ergosterol by mouth 10 hours previously. Various organs of the dogs also exhibited the vitamin, particularly the liver, adrenals, lungs, spleen, and mesentery, and sometimes the kidneys. No vitamin D was found in the heart, skin, brain and pancreas.

A considerable amount of the vitamin D ingested by animals appears in the feces. Hart, Steenbock, Kline and Humphrey (1930) detected it in the feces of cows given irradiated yeast. Hess, Light, Frey and Gross (1932) estimated that a fourth of the vitamin administered to cows as irradiated yeast appeared in the feces, but none in the urine.

Klein and Russell (1931) found that about a fourth of the vitamin D administered to chickens as irradiated ergosterol appeared in the droppings, and an even larger percentage of the vitamin D of cod liver oil. Hess, Weinstock, and Gross (1933) found the amount of vitamin D in the feces of infants to be more or less proportional to the amount received.

It is not known whether the vitamin D which appears in the feces has failed to be absorbed, or has been absorbed and subsequently re-excreted. Although precise balance experiments have not been made with vitamin D, it seems probable that some of it is destroyed in the body. Coppens and Metz (1933b) have reported that vitamin D is destroyed *in vitro* by blood and by lung tissue, but not by liver tissue. The inactivating action of the lung might account for the difference in vitamin D content of mammalian and fish livers.

Windaus and Rygh (1928) observed that certain irradiated ergosterol esters were antiricketic, while others, notably those in which the acid component was alien to the animal body, were more or less without action until after they had been saponified. This would indicate either that the ineffective esters are not hydrolyzed in the gut and fail to be absorbed, or that they are absorbed as esters and fail to be hydrolyzed after absorption. The first explanation is the more probable one, especially in view of the studies by Schönheimer and Hummel (1931) on the absorption of cholesteryl oxalate.

Greaves and Schmidt (1933), in studies on bile fistula rats in negative calcium balance, found that such animals did not benefit by irradiated ergosterol which was given by mouth, although they could utilize it when it was given subcutaneously. But the addition of desoxycholic acid to the vitamin given by mouth enabled the rats to assimilate the vitamin. Hence it seemed that bile, particularly desoxycholic acid, serves as a carrier of irradiated ergosterol across the intestinal wall. Bile fistula animals have an increased need for the antiricketic factor, which may be explained on the basis that they suffer an abnormal loss of vitamin along with their wasted bile. In later work, Greaves and Schmidt (1934a) showed that jaundiced rats readily stored vitamin D which was given subcutaneously, but not that which was given by mouth. Thus the rôle of bile in the transport of the vitamin across the intestinal wall was convincingly demonstrated.

*Mode and site of action.* The early workers on rickets comprehended something which indeed is almost obvious, namely, that vitamin D in some manner regulates the passage of Ca and P across the intestinal wall.

Such advances in knowledge and opinion as have been made are well stated by Harris and Innes (1931) and Harris (1932). These investigators hold the view that vitamin D exerts its action by raising the blood calcium and/or phosphate. This is associated usually with an increased net absorption from the gut, though under certain circumstances the bones may provide the calcium and phosphate. The net retention of the animal as a whole is the resultant of two opposing factors: 1, increased absorption from, or diminished excretion to the gut, and 2, increased excretion by the kidney. As the dosage of vitamin D becomes larger and larger, the second factor overtakes the first. This picture is, of course, more a statement of facts than a clarification of mechanisms.

In rickets the fecal pH is elevated, but tends to return to normal when vitamin D is administered (Zucker and Matzner, 1924; Abrahamson and Miller, 1925; Jephcott and Bacharach, 1926, 1928; Redman, Willimott, and Wokes, 1927; Yoder, 1927; Grayzel and Miller, 1928; Harris and Moore, 1929; Redman, 1929; Bacharach and Jephcott, 1929; Harris and Innes, 1931; and others). Because of this, the opinion was once offered that the action of the vitamin is local in the gut. However, in view of the fact that vitamin D circulates in the body, it would seem unlikely that such is the case.

There are at least two instances in which the action of vitamin D appeared to be elsewhere than in the gut: v. Brand and Holtz (1929, 1931) made subcutaneous injections of concentrated oily solutions of irradiated ergosterol, and observed that calcium deposits occurred at the site of injection. Local injury may, of course, have been a factor in this effect. Weinmann (1933) filled cavities prepared in the teeth of dogs with vitamin D, and observed that in 3 months a layer of new, over-calcified dentine developed. The results of the application of this discovery to man are awaited with impatience.

Another hypothesis is that vitamin D acts through stimulating the parathyroid glands (Taylor, Weld, Branion and Kay, 1931; and others). Although it is true that changes in the parathyroids are observed in rickets, and that either parathormone or irradiated ergosterol can produce hypercalcemia, metastatic calcifications, and other visceral injuries, nevertheless the effects of the two agents on the plasma phosphatase, on the blood and urine chemistry, and on the histology and chemical composition of the bones are dissimilar (Selye, 1932; Shelling, 1932a; Shelling, Asher and Jackson, 1933). Parathormone, although it relieves the tetany associated with rickets in infants, fails to induce healing in the ricketic metaphysis, and may actually retard it; whereas

vitamin D promotes calcification in the metaphysis, and in so doing may even temporarily induce hypocalcemia and tetany unless a sufficient amount of calcium is provided. The fact that vitamin D in large doses is able to raise the serum calcium in parathyroidectomized dogs and men, and thereby relieve their tetany has been attributed to stimulation of accessory parathyroid tissues, but the fact that the same relief is obtained in rats, in which parathyroid rests are probably rare, speaks against the view that vitamin D acts primarily through the parathyroids. Other evidence against such a direct relationship is seen in the effectiveness of vitamin D in animals and patients "immune" to the parathyroid hormone (Pugsley, 1932; Reed and Seed, 1933), and in the fact that parathyroidectomized and normal animals show little difference in susceptibility to toxic doses of irradiated ergosterol (Dale, Marble and Marks, 1932; Jones, 1933). Alleviation of parathyroid tetany was also brought about by the toxic, non-vitamin product which is produced by heating irradiated ergosterol (Popoviciu and Nitzescu, 1932; *cf.* Snapper, 1934), yet pure calciferol has been shown to be likewise capable of raising the blood calcium of parathyroidectomized dogs (Dale, Marble and Marks, 1932).

The review by Thomson and Collip (1932) is available to workers interested in the physiology of the parathyroid glands. Another review, by Shelling, dealing particularly with the relations between the parathyroids and vitamin D, is in preparation, and should serve to bring this involved subject up to date.

Small doses of vitamin D, *i.e.*, ordinary therapeutic doses, not only correct all the derangements of blood and tissue chemistry that are associated with rickets, but they may exert certain actions on animals not suffering from obvious, florid, rickets. Thus Kramer and Howland (1932) observed that deviations in Ca and P in the serum, which were produced by disproportion of these elements in the diet, were lessened by the administration of vitamin D. Templin and Steenbock (1933a, b) found that rats kept for long periods on a low-calcium, vitamin D-free ration showed loss of weight, lowered blood calcium and bone ash, premature senility, hypertrophied parathyroids, diestrus, and dental defects. These changes were largely prevented by the inclusion in the diet of a few units daily of vitamin D.

It is frequently assumed that the action of large doses of vitamin D is fundamentally the same, so far as mechanism is concerned, as that of small doses. Justifiably or otherwise, this assumption lies back of most experiments in which knowledge of the mechanism of ordinary

doses is sought in the results of the administration of massive, or at least generous, amounts of vitamin D. Large doses, perhaps in some experiments unnecessarily large, produce a number of effects other than toxication, which are listed here even though they shed no great light on the problem of mechanism.

Phillips and Robertson (1929), Corson, Irwin and Phillips (1930), and Phillips, Robertson, Corson and Irwin (1931) found that massive doses greatly increased the thrombocyte count of the blood of rats, and decreased the coagulation time, within a few days after administration. Crimm and Strayer (1934a) found that massive doses increased both the diffusible and the non-diffusible fractions of calcium in human blood, the non-diffusible form being the more affected. Crimm, Strayer, Watson, and Heimann (1933) reported that soon after the administration, there is a decrease in urinary and fecal calcium, but continued administration results in an increase in urinary calcium, still with a decrease in fecal calcium. The increased urinary calcium, which many authors have noted, is presumably related to the production of renal and bladder stones which have occasionally been associated with overdosage (Dixon and Hoyle, 1928; Harris and Moore, 1929; Hoyle and Buckland, 1929; Hoyle, 1930a). From the work of Crimm, *et al.*, there can be little doubt that when calcium is available in the diet, vitamin D increases its absorption, as indicated by the elevation of serum and urinary calcium. Increased dosage of vitamin D increases the absorption and retention of calcium to the point of hypercalcemia. "A true physiological action of vitamin D can only be analyzed in the face of an optimal intake of calcium and phosphorus."

Crimm and Strayer (1934b) observed, with massive doses, that hypercalcemia occurred in spite of no increase in hydrogen ion concentration of the blood. The glucose and $CO_2$ also were not significantly altered. The chloride content was slightly decreased, and the urea nitrogen slightly increased. There was a marked increase in the total blood protein, the rise being due to the albumin fraction. This is in accord with the view that calcium is retained by protein, in this case, albumin. There is often a rise in blood phosphorus soon after the administration of vitamin D, but as hypercalcemia appears, the hyperphosphatemia declines, in keeping with the reciprocity between Ca and P. Rappaport and Reed (1933) observed that massive doses caused a lowering of the potassium level of the blood, and a diminution of its fluctuations in hay fever patients.

Reed, Thacker, Dillman and Welch (1933) found that large doses

increased the metabolic rate in dogs. The hypercalcemia was accompanied by an increase in volume of urine, as has been reported by others. No significant change in nitrogen metabolism could be detected. Nitschke (1932) noted that hedgehogs given irradiated ergosterol at the time of their winter sleep did not hibernate, but remained warm and active. The experiments of Hess, Poncher, Dale and Klein (1930) suggest that even moderately excessive doses stimulate the metabolic rate in children. Curiously enough, it was found by Harris and Moore (1929) that lethal doses always *lowered* the heart beat in rats.

Again it is plain that a recital of facts does not explain the mechanisms behind them. It was suggested by McGowan, Cunningham, and Auchinachie (1931) and McGowan (1933) that vitamin D acts in rickets by setting free from the body lipids a supply of inorganic phosphate, "the relative deficiency of which is regarded as the essential cause of rickets." This idea is not strongly supported by experiment, and it may involve some confusion between cause and effect, but nevertheless it is interesting in view of the observation of Kay and Guyatt (1933) that vitamin D elevates the phosphoric ester content of the blood in the healing of rickets. The ester is assumed by these workers to be an important substrate for the always-present bone phosphatase, and "a chronic defect of phosphorus uptake from the diet" is regarded as the major factor in the production of rickets in the rat.

It is becoming known, from studies such as those of Benjamin and Hess (1933a, b) and Benjamin (1933), that the number of forms in which calcium and phosphorus exist in blood serum is larger than had been supposed. These workers recognize at least 4 forms of calcium and 2 of phosphorus, namely—

*Calcium:*
- Diffusible calcium ion
- Diffusible Ca-P complex, adsorbable on $BaSO_4$
- Non-diffusible complex, adsorbable on $BaSO_4$
- Non-diffusible complex, non-adsorbable on $BaSO_4$ ("protein-bound")

*Phosphorus:*
- Diffusible phosphate ion
- Diffusible Ca-P complex, adsorbable on $BaSO_4$

In rickets, the partition of the several forms of calcium is profoundly altered, even though the total quantity may not change. Vitamin D given to rickety animals increases the *adsorbable* fractions of calcium, and the total and adsorbable phosphorus. It seems to be the diffusible, adsorbable, Ca-P complex which provides the substance for the calcifica-

tion of bone. Parathyroid hormone, unlike vitamin D, increases the ion-containing fractions.

One can regard the several forms of blood calcium as being in equilibrium not only with each other, but with bone as a reservoir. Ham (1932) argues that an explanation of the mechanism of toxic calcification would throw light on the site and mode of action of vitamin D in its normal relations. He succeeded in producing calcifications in the aorta, coronary vessels, and heart musculature of rats without previous degenerative changes, merely by administering sufficiently massive doses of irradiated ergosterol. This would indicate that the calcification resulted simply from the inability of the serum to hold its calcium, rather than from direct action of the vitamin upon the recipient tissues. But how can serum acquire more calcium than it can later retain? Ham suggests that during the attainment of hypercalcemia there is a shift of calcium from the diffusible to the non-diffusible form, backed by a flow of more calcium from the gut or bones to the diffusible state. Any influence now causing the non-diffusible form to break down would result in the diffusible form passing the precipitation point. In keeping with this hypothesis are the facts that calcium depositions are not always associated with hypercalcemia, and that depositions frequently occur on the downswing of the hypercalcemia curve. These somewhat speculative, but significant, suggestions are elaborated in subsequent papers by Ham and Portuondo (1933), Schour and Ham (1934), and Ham and Lewis (1934). Some investigators will feel that Ham interprets too much on the premise that vitamin D acts through the parathyroid hormone.

Confusion in this field is understandable when one contemplates the permutations and combinations which are possible in a system comprising several forms of calcium and phosphorus in the blood, a reservoir and depository of these elements in bone, a fluctuating intake and output of them in the diet and excretions, and a dumping-place for them in the soft tissues—all under the influence of many factors besides the parathyroid glands and the several forms of vitamin D. As for the hormone and the vitamin, which seem to be the major forces in the system, it is most satisfactory to regard them as acting together—complementing, supplementing, or opposing each other as the occasion demands.

## REFERENCES

Numerous errors, due, perhaps, to cross-citation, are conspicuous in all textbook bibliographies dealing with the early investigations on the sterols. For the following compilation of over 600 references, the original publication has in each instance been examined.

ABDERHALDEN, E. Handbuch der biologischen Arbeitsmethoden. In many volumes. Urban & Schwarzenberg, Berlin and Wien. 1925 *et seq.*

ABDERHALDEN, E. AND E. R. LE COUNT. Ztschr. exp. Path. Therap. **2**: 199–215, 1906. Die Beziehungen zwischen Cholesterin, Lecithin und Cobragift, Tetanustoxin, Saponin und Solanin.

ABRAHAMSON, E. M. AND E. G. MILLER, Jr. Proc. Soc. Exp. Biol. Med. **22**: 438–439, 1925. Hydrogen-ion concentration in the gastro-intestinal tract of the albino rat.

ADAM, A. Klin. Wchnschr. **7**: 1825–1828, 1928. Zur Frage der Rachitisprophylaxe.

ALLEN, A. H. AND W. THOMSON. Chem. News **43**: 267, 1881. On the action of solvents on saponified oils and waxes.

American Medical Association, Council on Pharmacy and Chemistry. New and Nonofficial Remedies, Revised Supplement, 21–24. Viosterol. See also: J. A. M. A. **93**: 693, 1929. Viosterol.

American Medical Association, Council on Pharmacy and Chemistry. New and Nonofficial Remedies, 410–411, 1930. Viosterol.

American Medical Association, Council on Pharmacy and Chemistry. New and Nonofficial Remedies, 414–416, 1931. Viosterol.

American Medical Association, Council on Pharmacy and Chemistry. J. A. M. A., **102**: 1298–1300, 1934. Annual meeting of the Council on Pharmacy and Chemistry.

ANDERSON, R. J. AND E. CHARGAFF. J. Biol. Chem. **84**: 703–717, 1929. The chemistry of the lipoids of tubercle bacilli. V. Analysis of the acetone-soluble fat.

ANDERSON, R. J. AND M. G. MOORE. J. Am. Chem. Soc. **45**: 1944–1953, 1923. A study of the phytosterols of corn oil, cottonseed oil, and linseed oil.

ANDERSON, R. J., F. P. NABENHAUER AND R. L. SHRINER. J. Biol. Chem. **71**: 389–399, 1927. The distribution of dihydrositosterol in plant fats.

ANDERSON, R. J. AND R. L. SHRINER. J. Am. Chem. Soc. **48**: 2976–2986, 1926. The phytosterols of corn oil.

ANDERSON, R. J., R. L. SHRINER AND G. O. BURR. J. Am. Chem. Soc. **48**: 2987–2996, 1926. The phytosterols of wheat germ oil.

ANDRÉ, E., AND R. LECOQ. Compt. rend. **194**: 912–914, 1932. Sur les réserves en vitamines A et D de quelques poissons cartilagineux.

ANDREWS, E., R. SCHOENHEIMER AND L. HRDINA. Proc. Soc. Exp. Biol. Med. **28**: 945–946, 1931a. The etiology of gall stones. II. Rôle of the gall bladder.

Proc. Soc. Exp. Biol. Med. **28**: 947–948, 1931b. Etiology of gall stones. III. Bile salt-cholesterol ratio in human gall stone cases.

Arch. Surg. **25**: 796–810, 1932. Etiology of gallstones. I. Chemical factors and the rôle of the gallbladder.

ANGUS, T. C., F. A. ASKEW, R. B. BOURDILLON, H. M. BRUCE, R. K. CALLOW, C. FISCHMANN, J. ST. L. PHILPOT AND T. A. WEBSTER. Proc. Roy. Soc. London, series B, **108**: 340–359, 1931. A crystalline antirachitic substance.

ANITSCHKOW, N. Virchow's Arch. **249**: 73–82, 1924. Zur Ätiologie der Atherosklerose.

ANITSCHKOW, N. and S. CHALATOW. Centralbl. allg. Path. u. path. Anat. **24**: 1–9, 1913. Ueber experimentelle Cholesterinsteatose und ihre Bedeutung für die Entstehung einiger pathologischer Prozesse.

Annual reports on the progress of chemistry. Yearly. The Chemical Society, London.

ARNDT, H. J. Klin. Wchnschr. **5**: 1372–1373, 1926. Nahrungscholesterin-Blutcholesterin-Gallencholesterin.

ASKEW, F. A., R. B. BOURDILLON, H. M. BRUCE, R. K. CALLOW, J. ST. L. PHILPOT AND T. A. WEBSTER. Proc. Roy. Soc. London, series B, **109**: 488–506, 1932. Crystalline vitamin D.

ASKEW, F. A., R. B. BOURDILLON, H. M. BRUCE, R. G. C. JENKINS AND T. A. WEBSTER. Proc. Roy. Soc. London, series B, **107**: 76–90, 1930a. The distillation of vitamin D.

Proc. Roy. Soc. London, series B, **107**: 91–100, 1930b. The effects of further irradiation of the radiation products of ergosterol.

BACHARACH, A. L. AND H. JEPHCOTT. J. Biol. Chem. **82**: 751–758, 1929. Vitamin D and fecal reaction.

BAMBERGER, P. Deutsch. med. Wchnschr. **55**: 399–400, 1929. Zur Frage der Vigantolschäden.

BAMBERGER AND SPRANGER. Deutsch. med. Wchnschr. **54**: 1116–1119, 1928. Vigantol bei tuberkulösen Kindern.

BARR, D. P. Physiol. Rev. **12**: 593–624, 1932. Pathological calcification.

BAUMANN, E. J. AND O. M. HOLLY. J. Biol. Chem. **55**: 457–475, 1923. The relation of lipoids to suprarenal physiology. I. The cholesterol and lipoid phosphorus contents of the blood of rabbits before and after suprarenalectomy.

BEARD, H. H., R. E. BURK, H. E. THOMPSON AND H. GOLDBLATT. J. Biol. Chem. **96**: 307–312, 1932. The antirachitic activation of ergosterol in the absence of oxygen.

BECQUEREL, A. AND A. RODIER. Gaz. méd. de Paris, series 2, 12: 751–757, 765–772, 781–788, 797–802, 813–820, 1844a. Recherches sur la composition du sang dans l'état de santé et dans l'état de maladie.

Compt. rend. **19**: 1083–1088, 1844b. Recherches relatives à la composition du sang, dans l'état de santé et dans l'état de maladie.

Traité de chimie pathologique appliquée à la médecine pratique. 608 pp. Germer Baillière, Paris. 1854.

VON BEHRING, H. Ztschr. physiol. Chem. **192**: 112–113, 1930. Enthalten Bakterien Sterine?

BELLOC, G., R. FABRE AND H. SIMONNET. Compt. rend. **191**: 160–162, 1930. Contribution à l'étude de l'activité biologique des stérols. Étude des stérols de Plankton.

BENEKE, F. W. Studien über das Vorkommen, die Verbreitung und die Function von Gallenbestandtheilen in den thierischen und pflanzlichen Organismen. 144 pp. and 3 plates. Ricker, Giessen. 1862a.

Ann. **122**: 249–255, 1862b. Cholesterin im Pflanzenreich aufgefunden.

Arch. d. Vereins f. wissenschaftl. Heilk. **2**: 432–446, 1866. Ueber das Cholestearin.

BENEKE, F. W. Sitzungsberichte d. Gesellschaft zur Beförderung d. gesammten Naturwissensch. zu Marburg, 25–28, 1880. Ueber den Cholestearingehalt des menschlichen Gehirns.

BENJAMIN, H. R. J. Biol. Chem. **100:** 57–78, 1933. The forms of the calcium and inorganic phosphorus in human and animal sera. II. The nature and significance of the filtrable, adsorbable calcium-phosphorus complex.

BENJAMIN, H. R. AND A. F. HESS. J. Biol. Chem. **100:** 27–55, 1933a. The forms of the calcium and inorganic phosphorus in human and animal sera. I. Normal, rachitic, hypercalcemic, and other conditions.

J. Biol. Chem. **103:** 629–641, 1933b. The forms of the calcium and inorganic phosphorus in human and animal sera. III. A comparison of physiological and experimental hypercalcemia.

BERGMANN, W. J. Biol. Chem. **104:** 317–328, 1934a. Contributions to the study of marine products. II. The sterols of mollusks.

J. Biol. Chem. **104:** 553–557, 1934b. Contributions to the study of marine products. III. The chemistry of ostreasterol.

BERNAL, J. D. Nature **129:** 277–278, 1932. Crystal structures of vitamin D and related compounds.

J. Soc. Chem. Ind. **52:** 11, 1933a. Discussion.

J. Soc. Chem. Ind. **52:** 288, 1933b. Discussion.

BERTHELOT. Ann. chim. phys., series 3, **56:** 51–98, 1859. Sur plusieurs alcools nouveaux. Combinaisons des acides avec la cholestérine, l'éthal, le camphre de Bornéo et la méconine.

BETHKE, R. M., D. C. KENNARD AND H. L. SASSAMAN. J. Biol. Chem. **72:** 695–706, 1927. The fat-soluble vitamin content of hen's egg yolk as affected by the ration and management of the layers.

BETHKE, R. M., P. R. RECORD AND D. C. KENNARD. J. Nutr. **6:** 413–425, 1933. The comparative antirachitic efficiency of irradiated ergosterol, irradiated yeast, and cod liver oil for the chicken.

BEUMER, H. Deutsch. med. Wchnschr. **51:** 230, 1925. Gibt es einen intermediären Cholesterinabbau?

BILLS, C. E. Studies on the antiricketic vitamin. Dissertation, Johns Hopkins, 69 pp. 1924.

J. Biol. Chem. **66:** 451–457, 1925. Antiricketic substances. II. The action of *n*-butyl nitrite on activated cholesterol and the antiricketic vitamin.

J. Biol. Chem. **67:** 753–758, 1926. Antiricketic substances. III. The catalytic formation of an antiricketic cholesterol derivative.

Chem. Rev. **3:** 425–442, 1927a. The principal chemical researches on cod liver oil.

J. Biol. Chem. **72:** 751–758, 1927b. Antiricketic substances. VI. The distribution of vitamin D, with some notes on its possible origin.

BILLS, C. E. AND F. G. BRICKWEDDE. Nature **121:** 452, 1928. The activation of cholesterol at liquid oxygen temperature.

BILLS, C. E. AND E. M. HONEYWELL. J. Biol. Chem. **80:** 15–23, 1928. Antiricketic substances. VIII. Studies on highly purified ergosterol and its esters.

BILLS, C. E., E. M. HONEYWELL AND W. M. COX, JR. J. Biol. Chem. **80:** 557–563, 1928. Antiricketic substances. IX. Quantitative biophysical studies on the activation of ergosterol.

J. Biol. Chem. **92:** 601–604, 1931. Influence of solvents on the activation of ergosterol.

BILLS, C. E., E. M. HONEYWELL AND W. A. MACNAIR. J. Biol. Chem. **76:** 251–261 and 1 plate, 1928. Antiricketic substances. VII. Biochemical and spectrographic studies on purified cholesterol.

BILLS, C. E., E. M. HONEYWELL, A. M. WIRICK AND M. NUSSMEIER. J. Biol. Chem. **90:** 619–636, 1931. A critique of the line test for vitamin D.

BILLS, C. E. AND F. G. MCDONALD. J. Biol. Chem. **68:** 821–831 and 3 plates, 1926. Antiricketic substances. IV. The polymerization of cholesterol.

J. Biol. Chem. **72:** 1–11, 1927. The catalytic formation of mixed cholesteryl ethers.

BILLS, C. E., F. G. MCDONALD, L. N. BEMILLER, G. E. STEEL AND M. NUSSMEIER. J. Biol. Chem. **93:** 775–785, 1931. Heat of combustion of activated ergosterol.

BILLS, C. E., O. N. MASSENGALE AND P. S. PRICKETT. J. Biol. Chem. **87:** 259–264, 1930. Factors determining the ergosterol content of yeast. I. Species.

BILLS, C. E. AND G. E. STEEL. Proc. Soc. Exp. Biol. Med. **31:** 134–135, 1933. Ceryl alcohol from the grass, Agrostis.

BILLS, C. E. AND A. M. WIRICK. J. Biol. Chem. **86:** 117–128, 1930. Long time feeding experiments with activated ergosterol. I.

BIRKINSHAW, J. H., R. K. CALLOW AND C. F. FISCHMANN. Biochem. J. **25:** 1977–1980, 1931. Studies in the biochemistry of microorganisms. XXII. The isolation and characterization of ergosterol from *Penicillium puberulum* Bainier grown on a synthetic medium with glucose as sole source of carbon.

BLOOR, W. R. J. Biol. Chem. **25:** 577–599, 1916a. The distribution of the lipoids ("fat") in human blood.

J. Biol. Chem. **26:** 417–430, 1916b. The lipoids ("fat") of the blood in diabetes.

BLOOR, W. R. AND A. KNUDSON. J. Biol. Chem. **29:** 7–13, 1917. Cholesterol and cholesterol esters in human blood.

BLUNT, K. AND R. COWAN. Ultraviolet light and vitamin D in nutrition. 229 pp. University of Chicago Press, Chicago. 1930.

BOEHM, R. Biochem. Ztschr. **33:** 474–479, 1911. Ein Beitrag zur Chemie des Darminhaltes.

BONDZYŃSKI, S. Ber. **29:** 476–478, 1896. Ueber das Cholesterin der menschlichen Faeces.

BONDZYŃSKI, S. AND V. HUMNICKI. Ztschr. physiol. Chem. **22:** 396–410, 1896. Ueber das Schicksal des Cholesterins im thierischen Organismus.

BOUDET, F. Ann. chim. phys., series 2, **52:** 337–348, 1833a. Nouvelles recherches sur la composition du sérum du sang humain. J. pharm. chim., series 2, **19:** 475–481, 1833b. Essai critique et chimique sur le sang.

BOURDILLON, R. B. AND H. M. BRUCE. Biochem. J., **26**: 506–521 and 1 plate, 1932. The determination of vitamin D. II. A comparison of radiography and bone analysis in the estimation of vitamin D.

BOURDILLON, R. B., H. M. BRUCE, C. FISCHMANN AND T. A. WEBSTER. Medical Research Council, Special Report Series, no. 158. The quantitative estimation of vitamin D by radiography. 46 pp. and 2 plates. His Majesty's Stationery Office, London. 1931.

BOURDILLON, R. B., C. FISCHMANN, R. G. C. JENKINS AND T. A. WEBSTER. Proc. Roy. Soc. London, series B, **104**: 561–583 and 1 plate, 1929. The absorption spectrum of vitamin D.

BRACONNOT, H. Ann. chim. phys., series 1, **79**: 265–304, 1811. Recherches analytiques sur la nature des champignons.

v. BRAND, T. AND F. HOLTZ. Ztschr. physiol. Chem. **185**: 217–233 and 1 plate, 1929. Beitrag zum Verlauf und zur Prognose der durch Ueberdosierung von Vitamin-D-Lösungen erzeugten Krankheit.

Ztschr. physiol. Chem. **195**: 241–247, 1931. Ueber die lokale Gewebsverkalkung nach subcutaner Zufuhr von bestrahltem Ergosterin.

v. BRAND, T., F. HOLTZ AND W. PUTSCHAR. Arch. exp. Path. u. Pharm. **167**: 113–145, 1932. Vergleichende pharmakologische Untersuchungen über Calcinosefaktor und Nebenschilddrüsenhormon.

BRANION, H. D. AND J. B. SMITH. Poultry Sci. **11**: 261–265, 1932. The influence of vitamin D on hatchability and egg production.

BROWNING, E. The vitamins. 575 pp. Baillière, Tindall and Cox, London. 1931.

BRUCK-BIESOK, V., C. PIRQUET AND R. WAGNER. Klin. Wchnschr. **6**: 952, 1927. Rachitisprophylaxe.

BUKOVSKI, A. O sostavnîkh chastyakh plaunovavo masla (Oleum Lycopodii). Dissertation, Dorpat, 39 pp. 1889.

BUNKER, J. W. M., R. S. HARRIS AND R. S. EUSTIS. New Engl. J. Med. **208**: 313–315, 1933. The antirachitic potency of the milk of human mothers fed previously on "vitamin D milk" of the cow.

BURIÁN, R. Monatsh. **18**: 551–574, 1897. Ueber Sitosterin. (Ein Beitrag zur Kenntniss der Phytosterine.) Reprinted in Sitzber. Akad. Wiss. Wien, Math.-naturw. Classe, Abt. IIb., **106**: 549–572, 1897.

BUTENANDT, A. Ber. **64**: 2529–2539, 1931. Ueber das Pregnandiol, einen neuen Sterin-Abkömmling aus Schwangeren-Harn (2. Mitteil.); experimentell bearbeitet von F. Hildebrandt und H. Brücher.

Nature **130**: 238, 1932. Chemical constitution of the follicular and testicular hormones.

BUTENANDT, A., H. A. WEIDLICH AND H. THOMPSON. J. Soc. Chem. Ind. **52**: 289–290, 1933. Contribution to the constitution of the follicular hormone (oestrin).

CALLOW, R. K. Biochem. J. **25**: 79–86, 1931a. The purification of ergosterol. Biochem. J. **25**: 87–94, 1931b. Occurrence of $\alpha$-dihydroergosterol as an impurity in yeast ergosterol.

CALLOW, R. K. AND C. F. FISCHMANN. Biochem. J. **25**: 1464–1469, 1931. The occurrence of vitamin D in lampreys (Petromyzontidae).

CAVENTOU. J. pharm. chim., series 2, **11**: 462–465, 1825. Examen chimique de quelques productions animales morbides.

CHAMBERLAIN, E. N. Brit. Med. J. **2**: 896–898, 1929. The significance of cholesterol in physiology and pathology: a consideration of selected problems.

J. Physiol. **70**: 441–448, 1930. The effect of insulin and other endocrine extracts on the cholesterol content of tissues.

CHANNON, H. J. Biochem. J. **19**: 424–432, 1925. Cholesterol synthesis in the animal body.

CHEN, K. K. AND A. L. CHEN. J. Pharm. **49**: 514–525, 1933a. The physiological action of the principles isolated from the secretion of the Jamaican toad (Bufo marinus).

J. Pharm. **49**: 526–542, 1933b. A study of the poisonous secretions of five North American species of toads.

J. Pharm. **49**: 543–547, 1933c. The parotid secretion of B. bufo gargarizans as the source of Ch'an Su.

J. Pharm. **49**: 561–579, 1933d. Similarity and dis-similarity of bufagins, bufotoxins, and digitaloid glucosides.

CHEN, K. K., H. JENSEN AND A. L. CHEN. J. Pharm. **43**: 13–50, 1931. The pharmacological action of the principles isolated from Ch'an Su, the dried venom of the Chinese toad.

J. Pharm. **47**: 307–320, 1933a. The physiological action of the principles isolated from the secretion of the common European toad (Bufo bufo bufo).

J. Pharm. **49**: 14–25, 1933b. The physiological action of the principles isolated from the secretion of the European green toad (Bufo viridis viridis).

CHEVREUL. Ann. chim. phys., series 1, **95**: 5–50, 1815. Recherches chimiques sur plusieurs corps gras, et particulièrement sur leurs combinaisons avec les alcalis. V. Des corps qu'on a appelés adipocire, c'est-à-dire, de la substance cristallisée des calculs biliaires humains, du spermacéti et de la substance grasse des cadavres.

Ann. chim. phys., series 2, **2**: 339–372, 1816. Recherches chimiques sur les corps gras, et particulièrement sur leurs combinaisons avec les alcalis. VI. Examen des graisses d'homme, de mouton, de bœuf, de jaguar et d'oie.

Memoires du muséum d'histoire naturelle **11**: 239–240, 1824. Note sur la présence de la cholesterine dans la bile de l'homme. Reprinted in J. chim. med., series 1, **1**: 135–136, 1825.

CHICK, H. AND M. H. ROSCOE. Biochem. J. **20**: 632–649, 1926. Influence of diet and sunlight upon the amount of vitamin A and vitamin D in the milk afforded by a cow.

CHUANG, C. K. Ann. **500**: 270–280, 1933. Ueber die Konstitution des Ergosterins.

COLLAZO, J. A., P. RUBINO AND B. VARELA. Biochem. Ztschr. **204**: 347–353, 1929. Experimentelle Hypervitaminose der Ratten durch grosse Dosen bestrahlten Ergosterins.

COLLISON, D. L. AND I. S. MACLEAN. Biochem. J. **25**: 606–613, 1931. The nature of the lipoid matter extracted from green leaves (spinach and cabbage).

CONRADI, B. G. F. Dissertatio sistens experimenta nonnulla cum calculis vesiculae felleae humanae instituta. Jena, 1775. Cited by GREN, 1794. Reprinted in: Thesaurus dissertationum medicarum rariorum **1**: 191–211, 1784. Heidelberg. See pp. 200–206.

COOK, J. W. Proc. Roy. Soc. London, series B, **111**: 485–496 and 4 plates, 1932. The production of cancer by pure hydrocarbons. II.

COOK, J. W., E. C. DODDS AND C. L. HEWETT. Nature **131**: 56–57, 1933. A synthetic oestrus-exciting compound.

COOK, J. W. AND C. L. HEWETT. J. Soc. Chem. Ind. **52**: 451–452, 1933a. The dehydrogenation of cholesterol.

J. Chem. Soc. 1098–1111, 1933b. The synthesis of compounds related to the sterols, bile acids, and oestrus-producing hormones. Part I. 1:2-*cyclo*pentenophenanthrene.

COPPENS, P. A. AND G. A. METZ. Arch. néerland. physiol. **18**: 407–415, 1933a. Untersuchungen über die Verteilung des bestrahlten Ergosterins (D-Vitamins) im Tierorganismus nach Verabreichung per os.

Biochem. Ztschr. **266**: 169–174, 1933b. Die zerstörende Wirkung von Lungengewebe auf das antirachitische Vermögen bestrahlten Ergosterins (D-Vitamins).

CORSON, W. C., G. F. IRWIN AND R. A. PHILLIPS. Proc. Soc. Exp. Biol. Med. **27**: 488–490, 1930. The shortening of the coagulation time of the blood by irradiated ergosterol.

COUERBE, J. P. Ann. chim. phys., series 2, **56**: 160–193, 1834. Du cerveau, considéré sous le point de vue chimique et physiologique.

COWARD, K. H., F. J. DYER AND B. G. E. MORGAN. Analyst **57**: 368–372, 1932. The relative vitamin A and vitamin D content of samples of cod-liver oil.

COWARD, K. H. AND K. M. KEY. Biochem. J. **27**: 451–465, 1933. The degree of accuracy obtainable by the line test in estimations of vitamin D.

COX, W. M., JR. AND C. E. BILLS. J. Biol. Chem. **88**: 709–713, 1930. Antiricketic substances. X. On the relation of the isoergosterols to vitamin D.

CRIMM, P. D. Am. Rev. Tuberc. **26**: 112–123, 1932. Vitamine therapy in pulmonary tuberculosis. II. The treatment of pulmonary tuberculosis by means of hypercalcaemia induced by massive doses of activated ergosterol.

CRIMM, P. D. AND J. W. STRAYER. Am. J. Med. Sci. **187**: 557–562, 1934a. Vitamin therapy in pulmonary tuberculosis. V. The effect of Viosterol on the diffusible and non-diffusible calcium of the blood and spinal fluid.

J. Lab. Clin. Med. **19**: 966–971, 1934b. Vitamin therapy in pulmonary tuberculosis. VI. The effect of Viosterol on the carbon dioxide content, the H-ion concentration, chlorides, glucose and urea nitrogen of the blood, and protein, calcium, and phosphorus of the serum. The effect of physiologic saline on these constituents during the state of hypercalcemia.

CRIMM, P. D., J. W. STRAYER, H. L. WATSON AND G. HEIMANN. Am. Rev. Tuberc. **28**: 202–216, 1933. Vitamine therapy in pulmonary tuberculosis. III. The effect of Viosterol on the absorption, retention and excretion of calcium.

CURLING, T. B. A practical treatise on the diseases of the testis, and of the spermatic cord and scrotum. 568 pp. Carey and Hart, Philadelphia. 1843. See pp. 152–153.

CYTRONBERG, S. Biochem. Ztschr. **45**: 281–283, 1912. Ueber die Cholesterase der Blutkörperchen.

DALE, H., A. MARBLE AND H. P. MARKS. Proc. Roy. Soc. London, series B, **111**: 522–537, 1932. The effects on dogs of large doses of calciferol (vitamin D).

DALY, C. Proc. Soc. Exp. Biol. Med. **31**: 368–369, 1933. A note on the mode of action and administration of vitamin D.

DAM, H. Biochem. Ztschr. **232**: 269–273, 1931. Cholesterinbilanz des Hühnchens in den ersten zwei Lebenswochen.

DANIELLI, J. F., G. F. MARRIAN AND G. A. D. HASLEWOOD. Biochem. J. **27**: 311–320, 1933. Further observations on surface films of oestrin derivatives and of pregnandiol.

DEGKWITZ, R. Münch. med. Wchnschr. **75**: 1631–1634, 1928. Ueber die toxische Wirkung ultraviolettbestrahlter Milch und anderer Substanzen.

DEJUST, L. H., VAN STOLK AND M. E. DUREUIL. Compt. rend. **187**: 311–313, 1928. Sur la présence de l'ergostérol dans le sang humain.

DELAPLACE, R. AND G. REBIÈRE. Compt. rend. **188**: 1169–1172, 1929. Irradiation de l'ergostérol: action des rayons ultraviolets du quartz et des rayons X mous.

DELEZENNE, C. AND S. LEDEBT. Compt. rend. **152**: 790–792, 1911. Action du venin de cobra sur le sérum de cheval. Ses rapports avec l'hémolyse.

DELIUS, H. F. De cholelithis. Observationes et experimenta. 26 pp., 1 plate, and explanatory table. Walther, Erlangen. 1782.

DENIS, P. S. Recherches expérimentales sur le sang humain, considéré a l'état sain. 358 pp. C. F. Denis, Commercy. See p. 110. 1830.

DEVANEY, G. M., H. E. MUNSELL AND H. W. TITUS. Poultry Sci. **12**: 215–222, 1933. Effect of sources of vitamin D on storage of the antirachitic factor in the egg.

DIETRICH, G. S. Dissertatio inauguralis medica continens duas observationes rariores circa calculos in corpore humano inventos. Prooemium xvi pp., text 116 pp., and 2 plates. Halle. 1788. See pp. 66–69.

DIXON, W. E. AND J. C. HOYLE. Brit. Med. J. **2**: 832–835, 1928. The effects of irradiated ergosterol in large doses.

DORÉE, C. Biochem. J. **4**: 72–106, 1909. The occurrence and distribution of cholesterol and allied bodies in the animal kingdom.

DORÉE, C. AND J. A. GARDNER. Proc. Roy. Soc. London, series B, **80**: 212–226, 1908. The origin and destiny of cholesterol in the animal organism. Part I. On the so-called hippocoprosterol.

DRUMMOND, J. C. AND E. R. GUNTHER. Nature **126**: 398, 1930. Vitamin content of marine plankton.

DUBOSC, A. Caoutchouc et Gutta-percha **13**: 8870–8873; 8882–8885; 8904–8907; 8946–8950; 8983–8990; 9013–9016; 9055–9064, 1916. Les cholestérols au point de vue scientifique et industriel.

DUGUID, J. B. J. Path. Bact. **33**: 697–711 and 3 plates, 1930a. Vitamin D sclerosis in the rat's aorta.

Lancet **2**: 983–985, 1930b. The toxicity of vitamin D.

DUGUID, J. B., M. M. DUGGAN AND J. GOUGH. J. Path. Bact. **33**: 353–362, 1930. The toxicity of irradiated ergosterol.

DYER, F. J. Quart. J. Pharm. Pharmacol. **4**: 503–516 and 1 plate, 1931. The standardisation of vitamin D by the line test.

ECKSTEIN, H. C. AND U. J. WILE. J. Biol. Chem. **69**: 181–186, 1926. The cholesterol and phospholipid content of the cutaneous epithelium of man.

ELLIS, G. W. AND J. A. GARDNER, Proc. Roy. Soc. London, series B, **81**: 129–132, 1909. The origin and destiny of cholesterol in the animal organism. IV. The cholesterol contents of eggs and chicks.

ENDER, F. Ztschr. Vitaminforsch. **2**: 241–249, 1933. Der Unterschied zwischen dem synthetischen und dem natürlich vorkommenden D-Vitamin hinsichtlich ihrer physikalischen und chemischen Eigenschaften.

FABRE, R. AND H. SIMONNET. Compt. rend. **188**: 424–426, 1929. Étude comparative de la valeur de l'essai biologique et de l'essai physique de l'ergostérol irradié.

FALKENHEIM, C., W. VÖLTZ AND W. KIRSCH. Klin. Wchnschr. **5**: 2071–2072, 1926. Quarzlampenbestrahlung von Kühen und antirachitische Wirksamkeit ihrer Milch.

v. FEHLING, H. Neues Handwörterbuch der Chemie. In 4 vols. F. Vieweg und Sohn, Braunschweig. 1876. See vol. ii, pp. 649–654.

FLINT, A. Am. J. Med. Sci., new series, **44**: 305–365 and 3 plates, 1862. Experimental researches into a new excretory function of the liver; consisting in the removal of cholesterine from the blood, and its discharge from the body in the form of stercorine. (The seroline of Boudet.)

DE FOURCROY. Ann. chim. phys., series 1, **3**: 242–252, 1789. Examen chimique de la substance feuilletée et cristalline contenue dans les calculs biliaires, et de la nature des concrétions cystiques cristallisées.

GAMBLE, J. L. AND K. D. BLACKFAN. J. Biol. Chem. **42**: 401–409, 1920. Evidence indicating a synthesis of cholesterol by infants.

GARDNER, J. A. AND F. W. FOX. Proc. Roy. Soc. London, series B, **92**: 358–367, 1921. On the origin and destiny of cholesterol in the animal organism. XII. On the excretion of sterols in man.

GARDNER, J. A. AND H. GAINSBOROUGH. Biochem. J. **19**: 667–671, 1925. Cholesterol secretion in the urine. I.

Biochem. J. **21**: 130–140, 1927a. Studies on the cholesterol content of normal human plasma. I.

Biochem. J. **21**: 141–147, 1927b. Studies on the cholesterol content of normal human plasma. II. The attraction of the proteins of plasma for sterols.

GARDNER, J. A. AND P. E. LANDER. Biochem. J. **7**: 576–587, 1913. On the cholesterol content of the tissues of cats under various dietetic conditions and during inanition.

GÉRARD, E. Compt. rend. **114**: 1544–1546, 1892. Sur les cholestérines végétales.

Compt. rend. **121**: 723–726, 1895a. Sur les cholestérines des cryptogames.

J. pharm. chim., series 6, **1**: 601–608, 1895b. Sur les cholestérines des cryptogames.

Compt. rend. **126**: 909–911, 1898. Sur les cholestérines des végétaux inferieurs.

GERSTENBERGER, H. J. AND A. J. HORESH. J. Nutr. **5**: 479–483, 1932. The treatment of rachitic infants with milk produced by cows fed irradiated ergosterol.

GMELIN, L. Hand-book of chemistry. Transl. by H. Watts for the Cavendish Society. In 18 vols. Harrison, London. 1871. See vol. **18**, pp. 107–119.

GOBLEY. J. pharm. chim., series 3, **9**: 5–19 and 81–91, 1846. Recherches chimiques sur le jaune d'œuf.

J. pharm. chim., series 3, **11**: 409–417; **12**: 5–12, 1847. Recherches chimiques sur le jaune d'œuf, deuxième mémoire présenté à l'Académie royale des sciences.

J. pharm. chim., series 3, **17**: 401–417; **18**: 107–119, 1850. Recherches chimiques sur les œufs de carpe.

J. pharm. chim., series 3, **19**: 406–421, 1851a. Recherches chimiques sur la laitance de carpe.

J. chim. méd., series 3, **7**: 577–579, 1851b. Matières grasses du sang.

J. pharm. chim., series 3, **21**: 241–254, 1852. Recherches chimiques sur les matières grasses du sang veineux de l'homme.

J. pharm. chim., series 3, **30**: 241–246, 1856a. Recherches sur la nature chimique et les propriétés des matières grasses contenues dans la bile.

J. pharm. chim., series 3, **29**: 81–91, 1856b. Recherches chimiques sur les champignons vénéneux, premier mémoire, lu à l'Académie impériale de médecine.

GOLDBLATT, H. Ergebn. allg. Path. u. path. Anat., 2. Abt. **25**: 58–491, 1931. Die neuere Richtung der experimentellen Rachitisforschung.

GOLDBLATT, H. AND K. M. SOAMES. Biochem. J. **17**: 446–453, 1923. Studies on the fat-soluble growth-promoting factor. I. Storage. II. Synthesis.

GORIS, A. Ann. Inst. Pasteur **34**: 497–546, 1920. Composition chimique du bacille tuberculeux.

GÖTTCHE, O. AND B. KELLNER. Arch. Kinderheilk. **94**: 129–145, 1931. Die Toxizität einiger Ergosterinpräparate.

GOUGH, J., J. B. DUGUID AND D. R. DAVIES. Brit. J. Exp. Path. **14**: 137–145, 1933. The renal lesions in hypervitaminosis D: Observations on the urinary calcium and phosphorus excretion.

GOWEN, J. W., J. M. MURRAY, M. E. GOOCH AND F. B. AMES. Science, **63**: 97–98, 1926. Rickets, ultra-violet light and milk.

GRAYZEL, D. M. AND E. G. MILLER, JR. J. Biol. Chem. **76**: 423–436, 1928. The pH of the contents of the gastrointestinal tract in dogs, in relation to diet and rickets.

GREAVES, J. D. AND C. L. A. SCHMIDT. Proc. Soc. Exp. Biol. Med. **29**: 373–377, 1932. Studies on calcium and phosphorus in bile-fistula dogs.

J. Biol. Chem. **102**: 101–112, 1933. The rôle played by bile in the absorption of vitamin D in the rat.

Univ. Calif. Pub. Physiol. **8**: 43–48, 1934a. Further experiments on the rôle played by bile in the absorption of vitamin D in the rat.

Univ. Calif. Pub. Physiol. **8**: 49–60, 1934b. The action of Viosterol in jaundiced rachitic rats.

GREN. Crells Beytr. zu den chem. Ann. **4**: 19–26, 1789. Zerlegung eines Gallensteins.

GUERARD, A. J. chim. méd., series 1, **1**: 399–401, 1825. Examen d'une substance trouvée dans un kyste hydatique du foie chez un hypocondriaque.

GYÖRGY, P. Klin. Wchnschr. **8**: 684–691, 1929. Sind die bisher üblichen Gaben von bestrahltem Ergosterin als überflüssig hoch zu bezeichnen?

GYÖRGY, P. AND G. POPOVICIU. Jahrb. Kinderheilk. **132**: 34–74, 1931. Untersuchungen über die experimentelle Rattenrachitis. V. Zur Toxizität bestrahlter Ergosterinpräparate.

HAM, A. AND B. C. PORTUONDO. Arch. Path. **16**: 1–14, 1933. Relation of serum calcium to pathologic calcifications of hypervitaminosis D.

HAM, A. W. Arch. Path. **14**: 613–626, 1932. Mechanism of calcification in the heart and aorta in hypervitaminosis D.

HAM, A. W. AND M. D. LEWIS. Arch. Path. **17**: 356–361, 1934. Experimental intimal sclerosis of the coronary arteries of rats.

HARRIS, L. J. Lancet **1**: 236, 1930. Hypervitaminosis D: calcium-phosphorus intake a determining factor.

Lancet **1**: 1031–1038, 1932. The mode of action of vitamin D. The "parathyroid" theory: clinical hypervitaminosis.

HARRIS, L. J. AND J. R. M. INNES. Biochem. J. **25**: 367–390 and 2 plates, 1931. The mode of action of vitamin D. Studies on hypervitaminosis D. The influence of the calcium-phosphate intake.

HARRIS, L. J. AND T. MOORE. Biochem. J. **22**: 1461–1477, 1928. "Hypervitaminosis" and "vitamin balance."

Biochem. J. **23**: 261–273, 1929. Hypervitaminosis and vitamin balance. II. The specificity of vitamin D in irradiated ergosterol poisoning. III. The pathology of hypervitaminosis D.

HART, E. B., H. STEENBOCK, O. L. KLINE AND G. C. HUMPHREY. J. Biol. Chem. **86**: 145–155, 1930. Dietary factors influencing calcium assimilation. XIII. The influence of irradiated yeast on the calcium and phosphorus metabolism of milking cows.

HART, E. B., H. STEENBOCK, S. LEPKOVSKY, S. W. F. KLETZIEN, J. G. HALPIN AND O. N. JOHNSON. J. Biol. Chem. **65**: 579–595, 1925. The nutritional requirement of the chicken. V. The influence of ultra-violet light on the production, hatchability, and fertility of the egg.

HAUCH, J. T. Proc. Soc. Exp. Biol. Med. **30**: 475–477, 1933. The effect of high doses of irradiated and non-irradiated ergosterol on the albino rat.

HAUSMANN, W. Beitr. chem. Physiol. Path. **6**: 567–580, 1905. Ueber die Entgiftung des Saponins durch Cholesterin.

HÉDON, E. Arch. intern. pharmacodynamie **8**: 381–407; **9**: 393–406, 1900–1901. Sur l'hémolyse par les glycosides globulicides et les conditions de milieu qui la favorisent ou l'empêchent.

HEIDUSCHKA, A. AND H. LINDNER. Ztschr. physiol. Chem. **181**: 15–23, 1929. Ueber den Ergosteringehalt der Hefe.

HEILBRON, I. M. Brit. J. Actinotherapy, **2**: 210–215, 1928. Vitamin D and its relation to the irradiation of foodstuffs.

HEILBRON, I. M., E. D. KAMM AND R. A. MORTON. J. Soc. Chem. Ind. **45**: 932, 1926. The absorption spectra of cholesterol and its possible biological significance with reference to vitamin D. I. Preliminary observations. Published *in extenso*, Biochem. J. **21**: 78–85, 1927. The absorption

spectrum of cholesterol and its biological significance with reference to vitamin D. I. Preliminary observations.
Biochem. J. **21**: 1279–1283, 1927. The absorption spectra of oils and oil constituents with special reference to pro-vitamin D.

HEILBRON, I. M. AND W. A. SEXTON. Nature **123**: 567, 1929. The occurrence of ergosterol in phytosterols.

HEILBRON, I. M., J. C. E. SIMPSON AND F. S. SPRING. J. Chem. Soc., 626–635, 1933. The structure of the sterols and bile acids.

HENTSCHEL, H. AND O. BACHMANN. Ztschr. ges. exp. Med. **71**: 744–754, 1930. Gesamtsterin- und Ergosterinbestimmungen in der Kuhmilch.

HENTSCHEL, H. AND L. SCHINDEL. Klin. Wchnschr. **9**: 262, 1930. Ergosterinnachweis in menschlicher Haut.

HENZE, M. Ztschr. physiol. Chem. **41**: 109–124, 1904. Spongosterin, eine cholesterinartige Substanz aus Suberites domuncula, und seine angebliche Beziehung zum Lipochrom dieses Tieres.
Ztschr. physiol. Chem. **55**: 433–445, 1908a. Chemische Untersuchungen an Octopoden.
Ztschr. physiol. Chem. **55**: 427–432, 1908b. Ueber Spongosterin, das Cholesterin aus Suberites domuncula.

HEPNER, E. Pflüger's Arch. **73**: 595–606, 1899. Ueber den Cholesteringehalt der Blutkörperchen.

HERZENBERG, H. Beitr. path. Anat. **82**: 27–56, 1929. Studien über die Wirkungsweise des bestrahlten Ergosterins (Vigantol) und die Beziehungen der von ihm gesetzten Veränderungen zur Arteriosklerose.

HESS, A. F. J. A. M. A. **81**: 15–17, 1923. The therapeutic value of egg yolk in rickets.
Trans. Am. Ped. Soc. **36**: 57–60, 1924. Experiments on the action of light in relation to rickets. Reprinted, Am. J. Dis. Children, **28**, 517–520.
Rickets osteomalacia and tetany. 485 pp. Lea and Febiger, Philadelphia. 1929.

HESS, A. F., C. E. BILLS AND E. M. HONEYWELL. J. A. M. A. **92**: 226–228, 1929. Antirachitic potency in relation to volume of oil in the liver of the cod.

HESS, A. F. AND J. M. LEWIS. J. A. M. A. **91**: 783–788, 1928a. Clinical experience with irradiated ergosterol.
Personal communication. 1928b.
J. A. M. A. **99**: 647–653, 1932. Milk irradiated by the carbon arc lamp. A clinical and laboratory study of rickets.
J. A. M. A. **101**: 181–184, 1933. An appraisal of antirachitics in terms of rat and clinical units.

HESS, A. F., J. M. LEWIS, F. L. MACLEOD AND B. H. THOMAS. J. A. M. A. **97**: 370–375, 1931. Antirachitic potency of the milk of cows fed irradiated yeast or ergosterol.

HESS, A. F., J. M. LEWIS AND H. RIVKIN. J. A. M. A. **94**: 1885–1889, 1930. Newer aspects of the therapeutics of Viosterol (irradiated ergosterol).

HESS, A. F., R. F. LIGHT, C. N. FREY AND J. GROSS. J. Biol. Chem. **97**: 369–377, 1932. A study of the milk, blood, and excreta of cows fed moderate and excessive amounts of irradiated yeast or ergosterol.

HESS, A. F., A. M. PAPPENHEIMER AND M. WEINSTOCK. Proc. Soc. Exp. Biol. Med. **20**: 14–16, 1922. A study of light waves in relation to their protective action in rickets.

HESS, A. F. AND G. C. SUPPLEE. Proc. Soc. Exp. Biol. Med. **27**: 609–610, 1930. The action of irradiated ergosterol on rats and chickens.

HESS, A. F. AND L. J. UNGER. J. A. M. A. **69**: 1583–1586, 1917. Prophylactic therapy for rickets in a negro community.

HESS, A. F. AND M. WEINSTOCK. Am. J. Dis. Children, **27**: 1–5, 1924a. Antirachitic effect of cod liver oil fed during the period of pregnancy or lactation.

J. A. M. A. **83**: 1558–1562, 1924b. Rickets as influenced by the diet of the mother during pregnancy and lactation.

J. Biol. Chem. **62**: 301–313, 1924c. Antirachitic properties imparted to inert fluids and to green vegetables by ultra-violet irradiation.

J. Biol. Chem. **64**: 181–191, 1925a. The antirachitic value of irradiated cholesterol and phytosterol. II. Further evidence of change in biological activity.

J. Biol. Chem. **64**: 193–201, 1925b. The antirachitic value of irradiated cholesterol and phytosterol. III. Evidence of chemical change as shown by absorption spectra.

Proc. Soc. Exp. Biol. Med. **23**: 407–408, 1926. Puffer fish oil; a very potent antirachitic; its elaboration by fish deprived of sunlight.

HESS, A. F., M. WEINSTOCK AND J. GROSS. Proc. Soc. Exp. Biol. Med. **30**: 1357–1358, 1933. A biologic assay of blood and feces of infants receiving various antirachitics.

HESS, A. F., M. WEINSTOCK AND F. D. HELMAN. J. Biol. Chem. **63**: 305–308, 1925. The antirachitic value of irradiated phytosterol and cholesterol. I.

HESS, A. F., M. WEINSTOCK AND E. SHERMAN. Proc. Soc. Exp. Biol. Med. **23**: 636–638, 1926. The production of antirachitic properties in human milk resulting from irradiation of the mother.

J. A. M. A. **88**: 24–26, 1927. Antirachitic properties developed in human milk by irradiating the mother.

HESS, J. H., H. G. PONCHER, M. L. DALE AND R. I. KLEIN. J. A. M. A. **95**: 316–323, 1930. Viosterol (irradiated ergosterol). Prophylactic and therapeutic dosage.

HESSE, H. Biochem. Ztschr. **268**: 304–313, 1934. Ueber den Einfluss gesteigerter Vitamin-D-Zufuhr auf den Glutathiongehalt des Blutes.

HESSE, O. Ann. **192**: 175–179, 1878. Ueber Phytosterin und Cholesterin.

HEYL, F. W. J. Am. Chem. Soc. **54**: 1074–1076, 1932. The sterols of ergot. III. The occurrence of an isomer of alpha-dihydro-ergosterol.

HEYL, F. W. AND O. F. SWOAP. J. Am. Chem. Soc. **52**: 3688–3690, 1930. The sterols of ergot. II. The occurrence of dihydro-ergosterol.

HOLMES, A. D. J. Am. Pharm. Assn. **20**: 588–594, 1931. Report of the vitamin assay committee of the American Drug Manufacturers' Association—twentieth annual meeting—May 1931.

HOLTZ, F. Arch. klin. Chir. **177**: 32–39, 1933. Die Behandlung der postoperativen Tetanie.

HOLTZ, F., J. GÜRSCHING AND H. KRAUT. Arch. exp. Path. u. Pharm. **174:** 51–62, 1933. Vergleichende pharmakologische Untersuchungen über Calcinosefaktor und Nebenschilddrüsenhormon. II. Beobachtungen am Menschen.

HOLTZ, F., F. LAQUER, H. KREITMAIR AND T. MOLL. Biochem. Ztschr. **237:** 247–275, 1931. Beiträge zur Kenntnis des Vitamins D. I. Die Wertbestimmung im Tierversuch.

HOLTZ, F. AND E. SCHREIBER. Ztschr. physiol. Chem. **191:** 1–22, 1930. Einige weitere physiologische Erfahrungen über das bestrahlte Ergosterin und seine Umwandlungsprodukte.

HONEYWELL, E. M. AND C. E. BILLS. J. Biol. Chem. **99:** 71–78, 1932. Cerevisterol, a sterol accompanying ergosterol in yeast.

J. Biol. Chem. **103:** 515–520, 1933. Cerevisterol: new notes on composition, properties, and relation to other sterols.

HOPPE-SEYLER, F. Medicinisch-chemische Untersuchungen **1:** 162–163, 1866. Ueber einige Bestandtheile der Maiskörner.

Medicinisch-chemische Untersuchungen **4:** 486–501, 1871. Ueber die chemische Zusammensetzung des Eiters.

HOU, H. C. Chinese J. Physiol. **2:** 345–380, 1928. Studies on the glandula uropygialis of birds.

Chinese J. Physiol. **3:** 171–182, 1929. Relation of the preen gland (glandula uropygialis) of birds to rickets.

Chinese J. Physiol. **4:** 79–92 and 1 plate, 1930a. Further observations on the relation of the preen gland of birds to rickets.

Chinese J. Physiol. **4:** 345–358, 1930b. The rôle of the skin in the cure of rickets by irradiation. II. The site of activation of the provitamin.

Chinese J. Physiol. **5:** 11–18, 1931. Relation of preen gland of birds to rickets. III. Site of activation during irradiation.

HOU, H. C. AND E. TSO. Chinese J. Physiol. **4:** 93–116 and 2 plates, 1930. The rôle of the skin in the cure of rickets by irradiation.

HOYLE, J. C. J. Pharm. **38:** 271–289, 1930a. The toxic effects of irradiated ergosterol.

J. Pharm. **40:** 351–372, 1930b. Further observations on the toxic effects of irradiated ergosterol.

HOYLE, J. C. AND H. BUCKLAND. Biochem. J. **23:** 558–565, 1929. Further observations on the effects of large doses of irradiated ergosterol.

HUGHES, J. S., L. F. PAYNE, R. W. TITUS AND J. M. MOORE. J. Biol. Chem. **66:** 595–600, 1925. The relation between the amount of ultra-violet light received by hens and the amount of antirachitic vitamin in the eggs produced.

HULDSCHINSKY, K. Deutsch. med. Wchnschr. **45:** 712–713, 1919. Heilung von Rachitis durch künstliche Höhensonne.

Ztschr. orthop. Chir. **39:** 426–451, 1920. Die Behandlung der Rachitis durch Ultraviolettbestrahlung. Dargestellt an 24 Fällen.

HUME, E. M., N. S. LUCAS AND H. H. SMITH. Biochem. J. **21:** 362–367, 1927. On the absorption of vitamin D from the skin.

HUMMEL, R. Ztschr. physiol. Chem. **185:** 105–115, 1929. Ueber die Beziehungen der Gallensäuren zum Nahrungscholesterin. II.

HUNT, H. M. New Engl. J. Med. **201:** 659–667, 1929. Cholesterol in blood of diabetics treated at the New England Deaconess Hospital.

HUNTER, J. E., R. A. DUTCHER AND H. C. KNANDEL. Poultry Sci. **11,** 239–240, 1932. Does vitamin A possess vitamin D-sparing properties when fed to growing chicks?

HÜRTHLE, K. Ztschr. physiol. Chem. **21:** 331–359, 1896. Ueber die Fettsäure-Cholesterin-Ester des Blutserums.

I. G. Farbenindustrie Aktiengesellschaft. Brit. Patent 296,093, Nov. 15, 1928. 3 pp. Improvements in the photochemical production of vitamin D from ergosterol.

JACOBS, W. A. Physiol. Rev. **13:** 222–245, 1933. The chemistry of the cardiac glucosides.

JACOBS, W. A. AND R. C. ELDERFIELD. J. Biol. Chem. **97:** 727–737, 1932. Strophanthin. XXVII. Ring III of strophanthidin and related aglucones.

JACOBS, W. A. AND E. E. FLECK. J. Biol. Chem. **97:** 57–61, 1932. Strophanthin. XXVI. A further study of the dehydrogenation of strophanthidin.

JANKAU, L. Arch. exp. Path. u. Pharm. **29:** 237–243, 1891. Ueber Cholesterin- und Kalkausscheidung mit der Galle.

JENDRASSIK, A. AND A. G. KEMÉNYFFI. Biochem. Ztschr. **189:** 180–190, 1927. Zur Kenntnis des D-Vitasterins. I. Ueber die Aktivierbarkeit des Cholesterins.

JEPHCOTT, H. AND A. L. BACHARACH. Biochem. J. **20:** 1351–1355, 1926. A rapid and reliable test for vitamin D.

Biochem. J. **22:** 60–62, 1928. The quantitative estimation of vitamin D.

JOELSON, J. J. AND E. SHORR. Arch. Int. Med. **34:** 841–866, 1924. The relation of the suprarenals to cholesterol metabolism.

JONES, J. H. J. Biol. Chem. **100:** 343–348, 1933. Relation of the parathyroid glands to the toxicity of irradiated ergosterol.

JONES, J. H. AND M. RAPOPORT. J. Biol. Chem. **93:** 153–166, 1931. Further observations on the relation of calcium and phosphorus intake to the hypercalcemia and hyperphosphatemia induced by irradiated ergosterol.

KAY, H. D. AND B. L. GUYATT. Nature **131:** 468–469, 1933. Experimental rickets as a phosphorus deficiency disease.

KERN, R., M. F. MONTGOMERY AND E. U. STILL. J. Biol. Chem. **93:** 365–380, 1931. The effect of large doses of irradiated ergosterol upon nitrogen, calcium, and phosphorus metabolism in rats.

KING, E. J., H. HULL AND G. E. HALL. Poultry Sci. **12:** 129–132, 1933. Calcium-phosphorus metabolism in the chicken. IV. Ergosterol requirement of growing chicks.

KING, H., O. ROSENHEIM AND T. A. WEBSTER. Biochem. J. **23:** 166–167, 1929. Vitamin D from sterols of mummified Egyptian brain.

KLAUSNER-CRONHEIM, I. Deutsch. med. Wchnschr. **56:** 1566–1567, 1930. Nierenschädigungen durch Vigantol.

KLEIN, D. AND W. C. RUSSELL. J. Biol. Chem. **93:** 693–704, 1931. The fate of the antirachitic factor in the chicken. I. The antirachitic factor balance in the growing chick.

KNUDSON, A. J. Biol. Chem. **32**: 337–346, 1917. Relationship between cholesterol and cholesterol esters in the blood during fat absorption.

KNUDSON, A. AND C. N. MOORE. J. Biol. Chem. **81**: 49–64 and 1 plate, 1929. Comparison of the antirachitic potency of ergosterol irradiated by ultra-violet light and by exposure to cathode rays.

KOCH, E. M., F. C. KOCH AND H. B. LEMON. J. Biol. Chem. **85**: 159–167 and 4 plates, 1929. Absorption spectra studies on cholesterol and ergosterol.

KOCH, F. C., E. M. KOCH AND I. K. RAGINS. J. Biol. Chem. **85**: 141–158, 1929. Fractionation studies on provitamin D.

KOEHNE, M. AND L. B. MENDEL. J. Nutr. **1**: 399–443, 1929. The utilization of fatty oils given parenterally.

KON, G. A. R. J. Chem. Soc., 1081–1087, 1933. Syntheses of polycyclic compounds related to the sterols. Part I.

KON, S. K. AND R. G. BOOTH. Biochem. J. **28**: 121–130, 1934. The vitamin D activity of butter. III. An attempt to elucidate the nature of the labile factor in butter antirachitic for the rat. The antirachitic potency of lard, olive oil, egg oil and the fatty acids of butters and lard.

KON, S. K., F. DANIELS AND H. STEENBOCK. J. Am. Chem. Soc. **50**: 2573–2581, 1928. The quantitative study of the photochemical activation of sterols in the cure of rickets. II.

KÖNIG, J. AND J. SCHLUCKEBIER. Ztschr. Nahr. Genussm. **15**: 641–661, 1908. Ueber den Einfluss des Futterfettes auf das Körperfett bei Schweinen mit besonderer Berücksichtigung des Verbleibs des Phytosterins.

KRAMER, B. AND J. HOWLAND. J. Nutr. **5**: 39–60, 1932. Factors which determine the concentration of calcium and of inorganic phosphorus in the blood serum of rats.

KRAMER, B., S. D. KRAMER, D. H. SHELLING AND M. J. SHEAR. J. Biol. Chem. **71**: 699–706, 1927. Cod liver oil concentrate. Its value as an antirachitic agent when injected subcutaneously.

KRAUSS, W. E. AND R. M. BETHKE. J. Biol. Chem. **92**: pp. x-xi, 1931. Effect on the vitamin D content of milk of feeding irradiated ergosterol to cows.

KRAUSS, W. E., R. M. BETHKE AND C. F. MONROE. J. Nutr. **5**: 467–477, 1932. The effect of feeding irradiated ergosterol to cows on the vitamin D content of milk.

KRAUSS, W. E., R. M. BETHKE AND W. WILDER. J. Dairy Sci. **16**: 549–555, 1933. The effect of feeding a cod-liver oil concentrate to cows on the vitamin D content of milk.

KREITMAIR, H. AND T. MOLL. Münch. med. Wchnschr. **75**: 637–639, 1928. Hypervitaminose durch grosse Dosen Vitamin D.

KRESLING, K. Centr. Bakt., 1. Abt. **30**: 897–909, 1901. Ueber die Fettsubstanz der Tuberkelbacillen.

KUSUMOTO, C. Biochem. Ztschr. **14**: 411–415, 1908. Ueber den Cholesteringehalt der Hundefäces bei gewöhnlicher Ernährung und nach Fütterung von Cholesterin.

KYES, P. Berl. klin. Wchnschr. **40**: 956–959 and 982–984, 1903. Ueber die Isolirung von Schlangengift-Lecithiden.

LAAS, E. Virchow's Arch. **278**: 346–364, 1930. Die Anfänge der Vigantolschädigung beim Kaninchen.

LAHOUSSE AND GONNARD. J. Physique et Radium, series 6, **10**: 114S-116S, 1929. Sur le spectre d'absorption de la vitamine antirachitique.

LAQUER, F. AND O. LINSERT. Klin. Wchnschr. **12**: 753–754, 1933. Die biologische Wirkung des Toxisterins.

LASSAIGNE, J. L. Ann. chim. phys., series 2, **9**: 324–329, 1818. Analyse de plusieurs calculs et concrétions trouvés dans différens animaux.

J. chim. méd., series 1, **1**: 269–271, 1825. Analyse de plusieurs productions pathologiques chez l'homme.

Ann. chim. phys., series 2, **62**: 222–223, 1836. Note sur une concrétion formée dans le ventricule droit du cerveau d'un cheval.

LAURENS, H. The physiological effects of radiant energy. 610 pp. Chemical Catalog Co., New York. 1933.

League of Nations, Health Organisation, Permanent Commission on Biological Standardisation. Report of the conference on vitamin standards, no. C. H. 1055 (1). 15 pp. Geneva. 1931.

LEBERT, H. Physiologie pathologique. In 2 vols. and atlas. Baillière, Paris. 1845. See vol. **2**: pp. 103 and 263, and atlas.

LECANU. J. pharm. chim., series 2, **15**: 1–5, 1829. Sur l'existence de la cholestérine dans l'huile de jaunes d'œufs.

LECANU, L. R. Ann. chim. phys., series 2, **67**: 54–70, 1838. Études chimiques sur le sang humain.

LEHMANN, C. G. Lehrbuch der physiologischen Chemie. Second ed., in 2 vols. Engelmann, Leipzig. 1850a. See vol. **1**: pp. 282–287.

Lehrbuch der physiologischen Chemie. Second ed., in 2 vols. Engelmann, Leipzig. 1850b. See vol. **2**: p. 374.

LEIGH-CLARE, J. L. Biochem. J. **21**: 368–372, 1927. A search for vitamin D in the diatom *Nitzschia closterium* (W. Sm.).

LEVENE, P. A. AND I. P. ROLF. J. Biol. Chem. **55**: 743–749, 1923. Lysolecithins and lysocephalins.

LÉVY-SOLAL, CHRISTOU AND J. DALSACE. Compt. rend. soc. biol. **95**: 552–553, 1926. Pouvoir antirachitique des huiles végétales vieillies irradiées et administrées par voie parentérale ou sous-cutanée.

LEWKOWITSCH, J. Chemical technology and analysis of oils, fats, and waxes. 4th ed., in 3 vols. Macmillan, London. 1909. See vol. 1: p. 200.

Chemical technology and analysis of oils, fats, and waxes. 6th ed., in 3 vols. Macmillan, London. 1921. See chiefly vol. 1.

LIFSCHÜTZ, I. Biochem. Ztschr. **129**: 115–127, 1922. Zur Kenntnis des Metacholesterins und seiner Nebenprodukte. III.

Ztschr. physiol. Chem. **55**: 1–7, 1908. Studien über den Ursprung und die Bildung des Cholesterins.

LIGHT, R. F., G. MILLER AND C. N. FREY. J. Biol. Chem. **84**: 487–494, 1929. Studies on the effects of overdosage of vitamin D.

LINDENMEYER, O. J. prakt. Chem. **90**: 321–331, 1863. Beiträge zur Kenntniss des Cholesterins.

LINSERT, O. Cited, in a footnote, by WINDAUS, LÜTTRINGHAUS, AND DEPPE, 1931.

LOEFFLER, K. Ztschr. physiol. Chem. **178**: 186–191, 1928. Ueber Beziehungen der Gallensäuren zum Nahrungscholesterin.

LOOFBOUROW, J. R. Bull. Basic Sci. Research **3**: 101–156, 1931. Vitamin D—a review. I. Ultra-violet absorption spectra in relation to vitamin D. Sections A and B.

LUDWIG, H. Arch. Pharm., series 2, **137**: 36, 1869. Vorkommen von Cholesterin im Mutterkorn.

LUMIÈRE, A. AND R. H. GRANGE. Compt. rend. soc. biol. **104**: 205–207, 1930a. Effet protecteur du cholestérol contre les chocs provoqués par les hémolytiques chimiquement définis.

Compt. rend., **191**: 423–425, 1930b. Action protectrice du cholestérol contre les chocs provoqués par les floculats.

MCCOLLUM, E. V. AND N. SIMMONDS. The newer knowledge of nutrition. 4th ed., 594 pp. Macmillan, New York. 1929.

MCCOLLUM, E. V., N. SIMMONDS, J. E. BECKER AND P. G. SHIPLEY. J. Biol. Chem. **53**: 293–312, 1922. Studies on experimental rickets. XXI. An experimental demonstration of the existence of a vitamin which promotes calcium deposition.

J. Biol. Chem. **65**: 97–100, 1925. Studies on experimental rickets. XXVI. A diet composed principally of purified foodstuffs for use with the "line test" for vitamin D studies.

Am. J. Dis. Children **33**: 230–243, 1927. Studies on experimental rickets. XXVIII. Does vitamin D pass into the milk?

MACCORQUODALE, D. W., H. STEENBOCK AND H. ADKINS. J. Am. Chem. Soc. **52**: 2512–2518, 1930. The preparation and antirachitic activation of some derivatives of ergosterol and cholesterol.

MCDONALD, F. G. AND O. N. MASSENGALE. J. Biol. Chem. **99**: 79–83, 1932. The antiricketic potency of eggs from hens receiving massive doses of activated ergosterol.

MCGOWAN, J. P. Biochem. J. **27**: 943–950, 1933. Further investigations into the fundamental nature of vitamin D action.

MCGOWAN, J. P., I. J. CUNNINGHAM AND D. W. AUCHINACHIE. Biochem. J. **25**: 1295–1303, 1931. On the fundamental nature of vitamin D action.

MACLEAN, I. S. Biochem. J. **22**: 22–26, 1928. The isolation of a second sterol from yeast-fat.

MACLEAN, I. S. AND D. HOFFERT. Biochem. J. **20**: 343–357, 1926. The carbohydrate and fat metabolism of yeast. III. The nature of the intermediate stages.

MACLEOD, F. L. J. Nutr. **2**: 517–530, 1930. The chemistry of vitamin D.

MCMASTER, P. D. J. Exp. Med. **40**: 25–42, 1924. Studies on the total bile. VI. The influence of diet upon the output of cholesterol in the bile.

MACQUER, P. J. Dictionnaire de chymie. 2nd ed., in 4 vols. Didot, Paris. 1778. See vol. **2**: p. 195.

MADSEN, T. AND H. NOGUCHI. Chem. Centralbl. **1**: 1265, 1905. Toxine und Antitoxine. Saponin-Cholesterin.

MARCHAND, R. F. J. prakt. Chem., series 1, **16**: 37–42, 1839. Ueber die Verbreitung des Gallenfettes im thierischen Körper.

Lehrbuch der physiologischen Chemie. 458 pp. Simion, Berlin. 1844. See p. 258.

MARSHALL, A. L. AND A. KNUDSON. J. Am. Chem. Soc. **52**: 2304–2314, 1930. The formation of vitamin D by monochromatic light.

MASSENGALE, O. N., C. E. BILLS AND P. S. PRICKETT. J. Biol. Chem. **94:** 213–219, 1931. Factors determining the ergosterol content of yeast. II. Carbohydrate sources.

MASSENGALE, O. N. AND M. NUSSMEIER. J. Biol. Chem. **87:** 423–426, 1930. The action of activated ergosterol in the chicken. II. The prevention of leg weakness.

MATHEWS, A. P. J. Biol. Chem. **14:** 465–467, 1913. An important chemical difference between the eggs of the sea urchin and those of the star-fish.

MAYER, A. AND G. SCHAEFFER. Compt. rend. **155:** 728–731, 1912. Composition chimique du sang et hémolyse.

Medical Research Council. Lancet **2:** 503–504, 1930. The antirachitic vitamin D. Announcement of a standard by the Medical Research Council.

Medical Research Council. Special Report Series, No. 167. Vitamins: a survey of present knowledge. 332 pp. His Majesty's Stationery Office, London. 1932.

MELLANBY, E. Medical Research Council, Special Report Series, No. 61. Experimental rickets. 78 pp. and 129 figs. His Majesty's Stationery Office, London. 1921.

MENDEL, L. B. AND C. S. LEAVENWORTH. Am. J. Physiol. **21:** 77–84, 1908. Chemical studies on growth. VI. Changes in the purine-, pentose- and cholesterol-content of the developing egg.

MENOZZI, A. Atti accad. Lincei, Rendiconti, series 5, **17:** 1 sem., 91–94, 1908. Richerche nel gruppo della colesterina. IV.

MENOZZI, A. AND A. MORESCHI. Atti accad. Lincei, Rendiconti, series 5, **19:** 1 sem., 126–129, 1910. Richerche nel gruppo della colesterina. VI. Ulteriori osservazioni sulla bombicesterina e sulla presenza di colesterina nelle crisalidi del baco da seta.

MENSCHICK, W. AND I. H. PAGE. Ztschr. physiol. Chem. **211:** 246–252, 1932. Ueber die Resorbierbarkeit des unbestrahlten Ergosterins. Ein Beitrag zur Methodik der quantitativen Ergosterinbestimmung.

Ztschr. physiol. Chem. **218:** 95–103, 1933. Der Abbau des Cholesterins durch den tierischen Organismus. II. Bilanzversuche an Carnivoren bei starker Cholesterinfütterung.

MIESCHER, F. Ber. **7:** 376–379, 1874. Das Protamin, ein neue organische Base aus den Samenfäden des Rheinlachses.

MINOVICI, S. Bull. soc. chim. biol. **9:** 1129–1164, 1927. Contribution à l'étude du cholestérol au point de vue chimique et physiologique.

MITCHELL, J. MCK., J. EIMAN, D. V. WHIPPLE AND J. STOKES, JR. Am. J. Pub. Health **22:** 1220–1229, 1932. Protective value for infants of various types of vitamin D fortified milk. A preliminary report.

MOORE, R. B. AND T. DEVRIES. J. Am. Chem. Soc. **53:** 2676–2681, 1931. The activation of ergosterol with radium emanation.

MORGAN, R. S. Biochem. J. **26:** 1144–1154, 1932. The determination of vitamin D in the line test by measurement.

MORGENROTH, J. AND K. REICHER. Berl. klin. Wchnschr. **44:** 1200–1203, 1907. Zur Kenntnis der durch Toxolecithide erzeugten Anämie und deren medikamentöser Beeinflussung.

MORIN. J. chim. méd., series 1, **1:** 276–281, 1825. Examen chimique d'un liquide fourni par une tumeur enkistée qui avait son siège dans l'abdomen.

MORTON, R. A., I. M. HEILBRON AND E. D. KAMM. J. Chem. Soc., 2000–2005, 1927. The absorption spectrum of ergosterol in relation to the photosynthetic formation of vitamin D.

MUELLER, J. H. J. Biol. Chem. **21**: 23–28, 1915a. The cholesterol metabolism of the hen's egg during incubation.

J. Biol. Chem. **22**: 1–9, 1915b. The assimilation of cholesterol and its esters. J. Biol. Chem. **27**: 463–480, 1916. The mechanism of cholesterol absorption.

MÜLLER, P. Ztschr. physiol. Chem. **29**: 129–135, 1900. Ueber die Reduction des Cholesterins zu Koprosterin im menschlichen Darmkanal.

MUSSEHL, F. E. AND C. W. ACKERSON. Poultry Sci. **9**: 334–338, 1930. Irradiated ergosterol as an antirachitic for chicks.

VON NÄGELI, C. AND O. LOEW. Ann. **193**: 322–348, 1878. Ueber die chemische Zusammensetzung der Hefe.

NELSON, E. M. AND R. WALKER. J. A. M. A. **98**: 1263–1265, 1932. Vitamin content of medicinal cod liver oils and related products.

NICOLA, O.F. F. Revista méd. Lat.-Amer. **19**: 358–385 and 479–516, 1934. Consideraciones físicoquímicas sobre la transformación fotoquímica del ergosterol en vitamina D.

NITSCHKE, A. Ztschr. ges. exp. Med. **82**: 227–235, 1932. Ueber die Beeinflussung des Winterschlafs durch bestrahltes Ergosterin.

NOMURA, T. Tôhoku J. Exp. Med. **4**: 677–684, 1924. Zur Frage der Cholesterase im Blutserum und den Organextrakten.

OBERMÜLLER, K. Ztschr. physiol. Chem. **15**: 37–48, 1890. Beiträge zur Kenntniss des Cholesterins.

OVERTON, E. Jahrb. wiss. Botanik **34**: 669–701, 1900. Studien über die Aufnahme der Anilinfarben durch die lebende Zelle.

Studien über die Narkose. 195 pp. G. Fischer, Jena. 1901.

PAGE, I. H. AND W. MENSCHICK. Biochem. Ztschr. **221**: 6–10, 1930. Ueber die Wirkung von Ergosterinacetatverfütterung bei Kaninchen.

Biochem. Ztschr. **231**: 446–459, 1931. Ueber das Vorkommen von Ergosterin im menschlichen Gehirn.

J. Biol. Chem. **97**: 359–368, 1932. The destruction of cholesterol by the animal organism.

PAGE, I. H. AND H. RUDY. Biochem. Ztschr. **220**: 304–326, 1930. Ueber die Fettsäureester des Cholesterins.

PANZER, T. Ztschr. physiol. Chem. **73**: 109–127, 1911. Beitrag zur Biochemie der Protozoen.

Ztschr. physiol. Chem. **78**: 414–419, 1912. Notizen ueber die chemische Zusammensetzung der Tuberkelbazillen.

PARK, E. A. Physiol. Rev. **3**: 106–163, 1923. The etiology of rickets.

PARK, E. A. AND M. M. ELIOT. Article on rickets in: L. G. PARSONS AND S. BARLING. Diseases of infancy and childhood. In 2 vols. Oxford Univ. Press, London. 1933. See vol. **1**, pp. 216–295.

PARK, E. A. AND J. HOWLAND. Bull. Johns Hopkins Hosp. **32**: 341–344 and 4 plates, 1921. The radiographic evidence of the influence of cod-liver oil in rickets.

PARKE, J. L. Medicinisch-chemische Untersuchungen **2**: 211–214, 1867. Ueber die chemische Constitution des Eidotters.

PASCUCCI, O. Beitr. chem. Physiol. Path. **6**: 543–551, 1905. Die Zusammensetzung des Blutscheibenstromas und die Hämolyse.

PATEY, D. H. Brit. Med. J. **1**: 866–869, 1933. Modern views on the mechanism of gall-stone formation.

PETERS, J. P. AND D. D. VAN SLYKE. Quantitative clinical chemistry. In 2 vols. Williams & Wilkins, Baltimore. 1931. See chiefly vol. **1**: "Interpretations".

PHILLIPS, R. A. AND D. F. ROBERTSON. Proc. Soc. Exp. Biol. Med. **26**: 639–640, 1929. Effect of irradiated ergosterol on mammalian thrombocyte counts.

PHILLIPS, R. A., D. F. ROBERTSON, W. C. CORSON AND G. F. IRWIN. Ann. Internal Med., new series, **4**: 1134–1143, 1931. The effect of irradiated ergosterol on the thrombocytes and the coagulation of blood.

PHISALIX, C. Compt. rend. **125**: 1053–1055, 1897. La cholestérine et les sels biliaires vaccins chimiques du venin de vipère.

POHL, R. Nachr. Ges. Wissensch. Goettingen, Math.-physik. Klasse, 142–145, 1926. Ueber das Absorptionsspektrum des antirachitisch wirksamen Cholesterins.

Nachr. Ges. Wissensch. Goettingen, Math.-physik. Klasse, 185–191, 1927. Ueber das Absorptionsspektrum des antirachitischen Provitamins und Vitamins.

POLLARD, A., A. C. CHIBNALL AND S. H. PIPER. Biochem. J. **25**: 2111–2122, 1931. The wax constituents of forage grasses. I. Cocksfoot and perennial ryegrass.

POPOVICIU, G. Jahrb. Kinderheilk. **132**: 286–302, 1931. Untersuchungen über die experimentelle Rattenrachitis. VI. Untersuchungen über die Wirkung des bestrahlten Ergosterins und seiner Umwandlungsprodukte auf den Serumphosphatgehalt.

POPOVICIU, G. AND NITZESCU, I. I. Ztschr. ges. exp. Med. **81**: 656–677, 1932. Zur Wirkung bestrahlter Ergosterinpräparate bei der parathyreopriven Tetanie. (Zugleich Beiträge zu den innersekretorischen Beziehungen des antirachitischen und toxischen Prinzipes.)

PORTER, A. E. Biochem. J. **10**: 523–533, 1916. The distribution of esterases in the animal body.

POULLETIER DE LA SALLE. *Circa* 1769. See: DE FOURCROY, 1789.

POULSON, E. Strahlentherapie **34**: 648–649, 1929. Ueber das Vorkommen des antirachitischen Vitamins in Fischleberölen.

POULSSON, E. AND F. ENDER. Skand. Arch. Physiol. **66**: 92–96, 1933. On the vitamin content of cod-liver oil, as influenced by the nutritional state of the cod.

POULSSON, E. AND H. LÖVENSKIOLD. Biochem. J. **22**: 135–141 and 1 plate, 1928. The quantitative determination of vitamin D.

POWER, F. B. AND A. H. SALWAY. J. Chem. Soc. **103**: 399–406, 1913. The identification of ipuranol and some allied compounds as phytosterol glucosides.

PREUSS, C. L. Tuberculorum pulmonis crudorum analysis chemica. Dissertation, Berlin, 42 pp. 1835. See pp. 24, 25, 38.

PRIBRAM, H. Biochem. Ztschr. **1**: 413–424, 1906. Beitrag zur Kenntnis des Schicksals des Cholesterins und der Cholesterinester im tierischen Organismus.

PRICKETT, P. S. AND O. N. MASSENGALE. J. Infect. Diseases **49**: 297–302, 1931. Mycobacterium. Ergosterol content of certain species and effect of ergosterol on their growth.

PRUESS, L. M., W. H. PETERSON AND E. B. FRED. J. Biol. Chem. **97**: 483–489, 1932. Isolation and identification of ergosterol and mannitol from Aspergillus fischeri.

PREUSS, L. M., W. H. PETERSON, H. STEENBOCK AND E. B. FRED. J. Biol. Chem. **90**: 369–384, 1931. Sterol content and antirachitic activatability of mold mycelia.

PRYDE, J. Nature **133**: 237–239, 1934. Recent developments of sterol chemistry in relation to biological problems.

PUGSLEY, L. I. J. Physiol. **76**: 315–328, 1932. The effect of parathyroid hormone and of irradiated ergosterol on calcium and phosphorus metabolism in the rat.

PUTSCHAR, W. Ztschr. Kinderheilk. **48**: 269–281, 1929. Ueber Vigantolschädigung der Niere bei einem Kinde.

RABINOWITCH, I. M. Arch. Int. Med. **43**: 363–371, 1929. The cholesterol content of blood plasma in diabetes mellitus.

RANDLES, F. S. AND A. KNUDSON. J. Biol. Chem. **66**: 459–466, 1925. Studies on cholesterol. I. Synthesis of cholesterol in the animal body.
J. Biol. Chem. **76**: 89–93, 1928. Studies on cholesterol. III. The relation of the suprarenal gland and the spleen to cholesterol metabolism.

RANSOM, F. Deutsch. med. Wchnschr. **27**: 194–196, 1901. Saponin und sein Gegengift.

RAPPAPORT, B. Z. AND C. I. REED. J. A. M. A. **101**: 105–109, 1933. Viosterol of high potency in seasonal hay fever and related conditions.

RAULIN, J. Ann. sci. nat. Botan., series 5, **11**: 93–299, 1869. Études chimiques sur la végétation.

REDMAN, T. Biochem. J. **23**: 256–260, 1929. The hydrogen ion concentration and the calcium and phosphorus content of the faeces of rachitic children.

REDMAN, T., S. G. WILLIMOTT AND F. WOKES. Biochem. J. **21**: 589–605, 1927. The pH of the gastro-intestinal tract of certain rodents used in feeding experiments, and its possible significance in rickets.

REED, C. I. J. A. M. A. **102**: 1745–1748, 1934. Symptoms of Viosterol overdosage in human subjects.

REED, C. I., L. M. DILLMAN, E. A. THACKER AND R. I. KLEIN. J. Nutr. **6**: 371–381, 1933. The calcification of tissues by excessive doses of irradiated ergosterol.

REED, C. I. AND L. SEED. Endocrinol. **17**: 136–148, 1933. The treatment of clinical tetany with irradiated ergosterol.

REED, C. I. AND E. A. THACKER. Am. J. Physiol. **96**: 21–27, 1931. The effect of intravenous and intraperitoneal injections of irradiated ergosterol.

REED, C. I., E. A. THACKER, L. M. DILLMAN AND J. W. WELCH. J. Nutr. **6**: 355–370, 1933. The effects of irradiated ergosterol on the metabolism of normal dogs.

REERINK, E. H. AND A. VAN WIJK. Biochem. J. **23**: 1294–1307, 1929. The vitamin D problem. I. The photochemical reactions of ergosterol.

REINITZER, F. Monatsh. **9**: 421–441, 1888. Beiträge zur Kenntniss des Cholesterins.

REKLING, E. Strahlentherapie **25**: 568–579, 1927. Untersuchungen über die Wirkungsart des Lichts bei experimenteller Rattenrachitis.

RICHTER-QUITTNER, M. Wien. Arch. inn. Med. **1**: 425–454, 1920. Ueber die Verteilung des Cholesterins und seiner Ester auf Blutkörperchen und Plasma unter physiologischen und pathologischen Verhältnissen.

RITTER, E. Ztschr. physiol. Chem. **34**: 461–480, 1902. Beiträge zur Kenntniss des Sitosterins.

RITTHAUSEN, H. J. prakt. Chem. **88**: 145–146, 1863. Chemische Notitzen. IV. Cholesterin im Fett des Weizens.

ROBIN, C. AND F. VERDEIL. Traité de chimie anatomique et physiologique. In 3 vols. Baillière, Paris. 1853. See chiefly vol. **3**: pp. 48–69.

ROFFO, A. H. Compt. rend. **180**: 1529–1530, 1925. Cholestérine et hémolyse.

ROSENHEIM, O. Biochem. J. **23**: 47–53, 1929. A specific colour reaction for ergosterol.

ROSENHEIM, O. AND H. KING. J. Soc. Chem. Ind. **51**: 464–466, 1932. The ring-system of sterols and bile acids.

J. Soc. Chem. Ind. **52**: 11–12, 1933. Discussion.

J. Soc. Chem. Ind. **53**: 196–200, 1934. The ring-system of sterols and bile acids. V. On the constitution of ergosterol and its irradiation products.

ROSENHEIM, O. AND T. A. WEBSTER. Lancet **1**: 1025–1026, 1925. Rickets and cholesterol.

Biochem. J. **20**: 537–544 and 1 plate, 1926a. The anti-rachitic properties of irradiated sterols.

J. Soc. Chem. Ind. **45**: 932, 1926b. Further observations on the photochemical formation of vitamin D. Published *in extenso*, Biochem. J. **21**: 127–129, 1927. The relation of cholesterol to vitamin D.

Lancet **1**: 306–307, 1927a. On the nature of the parent substance of vitamin D.

Lancet **2**: 622–625, 1927b. The photochemical production of vitamin D from ergosterol.

Biochem. J. **21**: 389–397, 1927c. The parent substance of vitamin D.

ROWAN, W. Nature **121**: 323–324, 1928. Bird feathers and the antirachitic vitamin D.

RUBINSTEIN, M. Compt. rend. soc. biol. **80**: 191–194, 1917. L'athérome expérimentale par ingestion de cholestérine.

RUSSELL, F. S. Nature **126**: 472, 1930. Vitamin content of marine plankton.

RUSSELL, W. C. AND D. KLEIN. Poultry Sci. **10**: 269–274, 1931. The vitamin A content of a ration and the antirachitic potency of irradiated ergosterol.

RUSSELL, W. C., M. W. TAYLOR AND D. E. WILCOX. J. Biol. Chem. **99**: 109–118, 1932. The fate of the antirachitic factor in the chicken. II. The effectiveness of the factor administered by mouth and intraperitoneally.

RUZICKA, L., L. EHMANN, M. W. GOLDBERG AND H. HÖSLI. Helv. Chim. Acta. **16:** 833–841, 1933. Polyterpene und Polyterpenoide. LXXXIV. Synthese des 1,2-cyclopentanophenanthrens, des $\alpha$- und des $\beta$-methylderivats desselben und des Chrysens.

SALWAY, A. H. J. Chem. Soc. **103:** 1022–1029, 1913. The synthetical preparation of the *d*-glucosides of sitosterol, cholesterol, and some fatty alcohols.

SANDQVIST, H. AND E. BENGTSSON. Ber. **64:** 2167–2171, 1931. Zur Frage der Bruttoformel des Sitosterins.

SANDQVIST, H. AND J. GORTON. Ber. **63:** 1935–1938, 1930. Stigmasterin und dessen empirische Formel.

SCARFF, R. W. J. Path. Bact. **30:** 647–650 and 1 plate, 1927. The production of experimental atheroma with cholesterol.

SCHEER, K. AND T. SANDELS. Münch. med. Wchnschr. **77:** 1543–1545, 1930. Bestrahlte Milch für stillende Mütter zur Rachitisprophylaxe.

SCHEUNERT, A. AND M. SCHIEBLICH. Biochem. Ztschr. **209:** 290–303, 1929. Zur Wertbestimmung von Vitamin-D-Präparaten. I.

SCHIFF, A. Virchow's Arch. **278:** 62–83, 1930. Die durch Vigantol erzeugbaren Gefässwandveränderungen und ihre Rückbildungsfähigkeit im Tierversuch.

SCHLUTZ, F. W. AND M. MORSE. Am. J. Dis. Children **30:** 199–209, 1925. Some spectroscopic observations on cod liver oil.

SCHMIDTMANN. Centralbl. allg. Path. path. Anat. **43:** 8, 1928. Fütterungsversuche an milzlosen Tieren.

SCHMIDT-NIELSEN, S. AND S. SCHMIDT-NIELSEN. Ztschr. physiol. Chem. **189:** 229–238, 1930. Der Gehalt der Knorpelfische an antirachitischem Vitamin.

SCHOENHEIMER, R. Science **74:** 579–584, 1931. New contributions in sterol metabolism.

SCHOENHEIMER, R. AND F. BREUSCH. J. Biol. Chem. **103:** 439–448, 1933. Synthesis and destruction of cholesterol in the organism.

SCHOENHEIMER, R. AND L. HRDINA. Proc. Soc. Exp. Biol. Med. **28:** 944–945, 1931. The etiology of gall stones. I. Chemical factors.

SCHÖNHEIMER, R. Biochem. Ztschr. **147:** 258–263, 1924. Ueber die Resorptionbeschleunigung des Cholesterins bei Anwesenheit von Desoxycholsäure.

Ztschr. physiol. Chem. **185:** 119–122, 1929. Versuch einer Sterinbilanz an der legenden Henne.

Ztschr. physiol. Chem. **211:** 65–68, 1932. Bestehen Beziehungen zwischen Ergosterin und der Aorten-Verkalkung bei menschlicher Atherosklerose?

Ztschr. f. klin. Med. **123:** 749–763, 1933. Ueber eine Störung der Cholesterin-Ausscheidung. (Ein Beitrag zur Kenntnis der Hypercholesterinämien.)

SCHÖNHEIMER, R. AND H. V. BEHRING. Ztschr. physiol. Chem. **192:** 102–111, 1930. Ueber die Bedeutung gesättigter Sterine im Organismus. VI. Ueber die Exkretion gesättigter Sterine.

SCHÖNHEIMER, R., H. V. BEHRING AND K. V. GOTTBERG. Ztschr. physiol. Chem. **208:** 77–85, 1932. Ist unbestrahltes Ergosterin resorbierbar?

SCHÖNHEIMER, R., H. v. BEHRING, R. HUMMEL AND L. SCHINDEL. Ztschr. physiol. Chem. **192**: 73–111, 1930. Ueber die Bedeutung gesättigter Sterine im Organismus.

SCHÖNHEIMER, R. AND H. DAM. Ztschr. physiol. Chem. **211**: 241–245, 1932. Ueber Ergosterin-Resorption bei der legenden Henne.

SCHÖNHEIMER, R. AND L. HRDINA. Ztschr. physiol. Chem. **212**: 161–172, 1932. Ueber Exkretion und Rückresorption im Dünndarm. Mit besonderer Berücksichtigung der Sterine.

SCHÖNHEIMER, R. AND R. HUMMEL. Ztschr. physiol. Chem. **192**: 114–116, 1931. Beitrag zur Frage nach der Ester-Resorption.

SCHOUR, I. AND A. W. HAM. Arch. Path. **17**: 22–39, 1934. Action of vitamin D and of the parathyroid hormone on the calcium metabolism as interpreted by studying the effect of single doses on the calcification of dentin.

SCHREINER, O. AND E. C. SHOREY. J. Am. Chem. Soc. **31**: 116–118, 1909. The presence of a cholesterol substance in soils; agrosterol.

SCHULTZ, J. H. Biochem. Ztschr. **42**: 255–261, 1912. Untersuchungen betreffend des Vorkommen eines cholesterinspaltenden Fermentes in Blut und Leber.

SCHULZE, E. Ber. **5**: 1075–1078, 1872. Ueber die Zusammensetzung des Wollfetts.

Ber. **6**: 251–254, 1873a. Ueber die Zusammensetzung des Wollfetts. II.

J. prakt. Chem., N. F., **7**: 163–178, 1873b. Ueber die Zusammensetzung des Wollfetts.

Ztschr. physiol. Chem. **14**: 491–521, 1890. Bilden sich Cholesterine in Keimpflanzen, welche bei Lichtabschluss sich entwickeln?

SCHULZE, E. AND J. BARBIERI. J. prakt. Chem., N. F., **25**: 159–180 and 458–462, 1882. Zur Kenntniss der Cholesterine.

SCHULZE, E. AND E. WINTERSTEIN. Ztschr. physiol. Chem. **43**: 316–319, 1904. Ueber das Verhalten des Cholesterins gegen das Licht.

Ztschr. physiol. Chem. **48**: 546–548, 1906. Ueber das Verhalten des Cholesterins gegen das Licht. II.

SELYE, H. Endocrinol. **16**: 547–558, 1932. On the stimulation of new bone-formation with parathyroid extract and irradiated ergosterol.

SÉNAC. Cited by DE FOURCROY, 1789.

SETZ, P. Ztschr. physiol. Chem. **215**: 183–192, 1933. Photochemische Unwandlungen der Bestrahlungsprodukte des Ergosterins.

SHELLING, D. H. J. Biol. Chem. **96**: 215–228, 1932a. Calcium and phosphorus studies. II. The effect of diet and of Viosterol on the tetany and on the serum calcium of parathyroidectomized rats.

J. Biol. Chem. **96**: 229–243, 1932b. Calcium and phosphorus studies. III. The source of excess serum calcium in Viosterol hypercalcemia.

SHELLING, D. H. AND D. E. ASHER. Bull. Johns Hopkins Hosp. **50**: 318–343, 1932. Calcium and phosphorus studies. IV. The relation of calcium and phosphorus of the diet to the toxicity of Viosterol.

SHELLING, D. H., D. E. ASHER AND D. A. JACKSON. Bull. Johns Hopkins Hosp. **53**: 348–389, 1933. Calcium and phosphorus studies. VII. The effects

of variations in dosage of parathormone and of calcium and phosphorus in the diet on the concentrations of calcium and inorganic phosphorus in the serum and on the histology and chemical composition of the bones of rats.

SHELLING, D. H. AND K. B. HOPPER. Am. J. Dis. Children **47**: 61–90, 1934. Calcium and phosphorus studies. VI. Observations on the treatment of late rickets with Viosterol based on the study of twenty-three cases.

SHIPLEY, P. G., E. A. PARK, E. V. MCCOLLUM, N. SIMMONDS AND H. T. PARSONS. J. Biol. Chem. **45**: 343–348 and 1 plate, 1921. Studies on experimental rickets. II. The effect of cod liver oil administered to rats with experimental rickets.

SHOPE, R. E. J. Biol. Chem. **80**: 127–132, 1928. Cholesterol esterase in animal tissues.

SIMON, J. F. Animal chemistry with reference to the physiology and pathology of man. Transl. by G. E. Day. 717 pp. Lea and Blanchard, Philadelphia. 1846. See pp. 572–573.

SMAKULA, A. Nachr. Ges. Wissensch. Goettingen, Math.-physik. Klasse, 49–64, 1928. Zur Kenntnis des Absorptionsspektrums von Ergosterin in bestrahltem und unbestrahltem Zustande.

SMITH, H. P. AND G. H. WHIPPLE. J. Biol. Chem. **80**: 671–684, 1928. Bile salt metabolism. II. Influence of meat and meat extractives, liver and kidney, egg yolk and yeast in the diet.

SNAPPER, I. Lancet, **1**: 728–731, 1934. The treatment of tetany.

SOAMES, K. M. Biochem. J. **18**: 1349–1353, 1924. A preliminary note on the growth-promoting and anti-rachitic value of cod liver oil when injected intraperitoneally.

SPIES, J. W. AND G. P. LYMAN. Am. J. Physiol. **102**: 527–533, 1932. The intraperitoneal administration of Viosterol in mice.

SPIES, T. D. Am. Rev. Tuberc. **23**: 169–174, 1931. The calcification of tubercles by means of irradiated ergosterol in experimental chronic tuberculosis.

SPIES, T. D. AND R. F. HANZAL. Proc. Soc. Exp. Biol. Med. **31**: 747–750, 1934. Experimental production of hypercalcemia in human beings by means of irradiated ergosterol.

STEENBOCK, H. Science **60**: 224–225, 1924. The induction of growth promoting and calcifying properties in a ration by exposure to light.

U. S. Patent 1,680,818, Aug. 14, 1928. 5 pp. Antirachitic product and process.

STEENBOCK, H. AND A. BLACK. J. Biol. Chem. **61**: 405–422, 1924. Fat-soluble vitamins. XVII. The induction of growth-promoting and calcifying properties in a ration by exposure to ultra-violet light.

J. Biol. Chem. **64**: 263–298, 1925. Fat-soluble vitamins. XXIII. The induction of growth-promoting and calcifying properties in fats and their unsaponifiable constituents by exposure to light.

STEENBOCK, H., E. B. HART, F. HANNING AND G. C. HUMPHREY. J. Biol. Chem. **88**: 197–214, 1930. Fat-soluble vitamins. XXX. The antirachitic value of cow's milk as modified by the feeding of irradiated yeast.

STEENBOCK, H., E. B. HART, C. A. HOPPERT AND A. BLACK. J. Biol. Chem. **66**: 441–449, 1925. Fat-soluble vitamin. XXVI. The antirachitic property of milk, and its increase by direct irradiation and by irradiation of the animal.

STEENBOCK, H., S. W. F. KLETZIEN AND J. G. HALPIN. J. Biol. Chem. **97**: 249–264, 1932. The reaction of the chicken to irradiated ergosterol and irradiated yeast as contrasted with the natural vitamin D of fish liver oils.

STEENBOCK, H. AND M. T. NELSON. J. Biol. Chem. **62**: 209–216 and 1 plate, 1924. Fat-soluble vitamins. XIX. The induction of calcifying properties in a rickets-producing ration by radiant energy.

STEPP, W. Ztschr. Biol. **83**: 102–106, 1925. Ueber die Wirksamkeit parenteraler Zufuhr der Vitamine A und D bei experimentellen Avitaminosen.

SUMI, M. Sci. Papers Inst. Phys. Chem. Research (Tokyo) **20**: 254–258, 1933. On the ergosterin content of various edible mushrooms in Japan.

SUPPLEE, G. C., G. E. FLANIGAN, O. J. KAHLENBERG AND A. F. HESS. J. Biol. Chem. **91**: 773–789 and 2 plates, 1931. The comparative antirachitic and calcifying properties of irradiated milk and milk derivatives.

SWEANY, H. C., A. T. WEATHERS AND K. L. MCCLUSKEY. Am. Rev. Tuberc. **8**: 405–437, 1924. The chemistry of the blood in tuberculosis.

TAMMANN, H. Beitr. klin. Chir. (Bruns') **142**: 83–120, 1928. Ueber die Beeinflussung der porotischen Osteomalazie nach Gallenfistel durch das D-Vitamin.

TANRET, C. Ann. chim. phys., series 5, **17**: 493–512, 1879. De l'ergotinine.

Compt. rend. **108**: 98–100, 1889. Sur un nouveau principe immédiat de l'ergot de seigle, l'ergostérine.

Ann. chim. phys., series 6, **20**: 289–297, 1890. Sur un nouveau principe immédiat de l'ergot de seigle: l'ergostérine.

Compt. rend. **147**: 75–77, 1908a. Sur l'ergostérine et la fongistérine.

Ann. chim. phys., series 8, **15**: 313–330, 1908b. Sur l'ergostérine et la fongistérine.

TANRET, G. Bull. Soc. Chim. biol. **15**: 1346–1364, 1933. De l'ergostérol irradié à la vitamine D cristallisée, d'après les travaux de Bourdillon et de Windaus.

TAYLOR, N. B., C. B. WELD, H. D. BRANION AND H. D. KAY. Can. Med. Assn. J. **24**: 763–777; **25**: 20–35, 1931. A study of the action of irradiated ergosterol and of its relationship to parathyroid function.

TEMPLIN, V. M. AND H. STEENBOCK. J. Biol. Chem. **100**: 209–216, 1933a. Vitamin D and the conservation of calcium in the adult. II. The effect of vitamin D on calcium conservation in adult rats maintained on low calcium diets.

J. Biol. Chem. **100**: 217–224, 1933b. Vitamin D and the conservation of calcium in the adult. III. The effect of vitamin D on the teeth of rats.

TERROINE, E. F., R. BONNET, G. KOPP AND J. VECHOT. Bull. Soc. Chim. biol. **9**: 678–691, 1927. La formation des stérols est-elle liée au métabolisme des matières grasse?

THANNHAUSER, S. J. Deutsch. Arch. klin. Med. **141**: 290–311, 1923. Ueber den Cholesterinstoffwechsel. (With Drs. Fleischmann, Andersen, Carius, Miller, Schaber and Moncorps).

THANNHAUSER, S. J. AND H. SCHABER. Ztschr. physiol. Chem. **127**: 278–280, 1923. Kann der tierische Organismus Cholesterin synthetisieren?

THOMAS, B. H. AND F. L. MACLEOD. Science **73**: 618–620, 1931. Increasing the vitamin D potency of cow's milk by the daily feeding of irradiated yeast or irradiated ergosterol.

THOMPSON, T. Proc. Roy. Soc. London **7**: 41–42, 1854. On the changes produced in the blood by the administration of cod-liver oil and cocoa-nut oil.

THOMS, H. Arch. Pharm. **235**: 39–43, 1897. Ueber Phytosterine.

THOMSON, D. L. AND J. B. COLLIP. Physiol. Rev. **12**: 309–383, 1932. The parathyroid glands.

TOLMATSCHEFF. Medicinisch-chemische Untersuchungen **2**: 272–278, 1867. Zur Analyse der Milch.

TULLY, W. C., S. M. HAUGE, C. W. CARRICK AND R. E. ROBERTS. Poultry Sci. **10**: 310–318, 1931. Calcium and phosphorus requirements for growing chicks. II. Studies with natural minerals under rachitic conditions.

U. S. P. X. Interim Revision Announcement no. 2. 1934. 1–11. A 1934 revision of the text and assays for cod liver oil of the Pharmacopœia of the United States. Tenth decennial revision.

VALLISNERI, A. Opere fisico-mediche. In 3 vols. Coleti, Venice. 1733. See vol. **3**: p. 594.

VAUQUELIN. Ann. chim. phys., series 1, **85**: 5–25, 1813. Expériences sur les champignons.

VOGEL, J. Anleitung zum Gebrauch des Mikroskopes zur zoochemischen Analyse und zur mikroskopisch-chemischen Untersuchung. 600 pp. and 3 plates. Voss, Leipzig. 1841. See pp. 293–295, 597, and fig. 3 of plate iii.

Erlaeuterungstafeln zur pathologischen Histologie. 128 pp. and 26 plates. Voss, Leipzig. 1843. See pp. 52, 101, 102, fig. 1 of plate xi, and fig. 7 of plate xxii.

The pathological anatomy of the human body. Transl. by G. E. Day. 534 pp. and 10 plates. Lea and Blanchard, Philadelphia. 1847. See p. 531 and fig. 1 of plate x.

WACHTEL, M. Münch. med. Wchnschr. **76**: 1513–1514, 1929. Die Vermehrung und Vitaminanreicherung der Muttermilch und Kuhmilch mittels bestrahlter Hefe.

WACKER, L. AND W. HUECK. Arch. exp. Path. u. Pharm. **74**: 416–441, 1913. Chemische und morphologische Untersuchungen über die Bedeutung des Cholesterins im Organismus. IV. Ueber den Cholesteringehalt des Blutes verschiedener Tiere und den Einfluss künstlicher Cholesterinzufuhr, besonders mit der Nahrung.

WADDELL, J. J. Biol. Chem. **105**: 711–739, 1934. The provitamin D of cholesterol. I. The antirachitic efficacy of irradiated cholesterol.

WATCHORN, E. Biochem. J. **24**: 631–640, 1930. The absorption and excretion of calcium and phosphorus by rats receiving excessive doses of irradiated ergosterol.

WEBSTER, T. A. AND R. B. BOURDILLON. Biochem. J. **22**: 1223–1230 and 2 plates, 1928. Notes on the irradiation of ergosterol.

Nature **123**: 244, 1929. The absorption spectrum of vitamin D.

WEIDLICH, G. Ztschr. Vitaminforsch. **2**: 253–266, 1933. Die Chemie des Vitamins D.

WEINMANN, J. J. Dental Res. **13**: 220–221, 1933. Action of vitamin D, by local application, in dental cavities.

WELSCH, A. Ueber das Vorkommen und die Verbreitung der Sterine im Thier- und Pflanzenreich. Dissertation, Freiburg im Breisgau, 59 pp., 1909.

WIELAND, H. AND R. ALLES. Ber. **55**: 1789–1798, 1922. Ueber den Giftstoff der Kröte.

WIELAND, H. AND M. ASANO. Ann. **473**: 300–313, 1929. Zur Kenntnis der Sterine der Hefe.

WIELAND, H. AND E. DANE. Ztschr. physiol. Chem. **210**: 268–281, 1932. Untersuchungen über die Konstitution der Gallensäuren.

WIELAND, H. AND G. A. C. GOUGH. Ann. **482**: 36–49, 1930. Zur Kenntnis der Sterine der Hefe. II.

WIELAND, H., G. HESSE AND H. MEYER. Ann. **493**: 272–280, 1932. Der Abbau des Bufotalins zu einer Cholansäure. Ueber Krötengiftstoffe. VI.

VAN WIJK, A. AND E. H. REERINK. Nature **122**: 648, 1928. Vitamin-D and isoergosterol.

WILKINS, L. AND B. KRAMER. Bull. Johns Hopkins Hosp. **40**: 52–57, 1927. Infantile rickets. Treatment by intramuscular injection of a cod-liver oil concentrate.

WINDAUS, A. Arch. Pharm. **246**: 117–149, 1908. Untersuchungen über Cholesterin.

Ber. **42**: 238–246, 1909. Ueber die Entgiftung der Saponine durch Cholesterin.

Ztschr. physiol. Chem. **65**: 110–117, 1910. Ueber die quantitative Bestimmung des Cholesterins und der Cholesterinester in einigen normalen und pathologischen Nieren.

Nachr. Ges. Wissensch. Goettingen, Math.-physik. Klasse, 301–304, 1916. Ueber das verschiedene physiologische Verhalten einiger stereoisomerer Alkohole der Cholesterinreihe.

Ann. **453**: 101–112, 1927. Umlagerung des Cholesterins.

Nachr. Ges. Wissensch. Goettingen, Math.-physik. Klasse, 36–57, 1930. Einige weitere Erfahrungen über das bestrahlte Ergosterin.

Deutsch. med. Wchnschr. **57**: 678–680, 1931a. Einige Bemerkungen über das bestrahlte Ergosterin.

Proc. Roy. Soc. London, series B, **108**: 568–575, 1931b. The chemistry of irradiated ergosterol.

Nachr. Ges. Wissensch. Goettingen, Math.-physik. Klasse, 408–417, 1932a. Uebergang von Sterinen in aromatische Verbindungen.

Ztschr. physiol. Chem. **213**: 147–187, 1932b. Ueber die Konstitution des Cholesterins und der Gallensäuren.

Nachr. Ges. Wissensch. Goettingen, Math.-physik. Klasse, 92–102, 1933. Einige Bemerkungen über die Konstitution des Cholesterins und des Ergosterins.

WINDAUS, A. AND E. AUHAGEN. Ztschr. physiol. Chem. **196**: 108–120, 1931a. Ueber die Beständigkeit des bestrahlten Ergosterins.

Ztschr. physiol. Chem. **197**: 167–172, 1931b. Vergebliche Versuche zur Abtrennung des antirachitischen vom toxischen Faktor in Bestrahlungsprodukten des Ergosterins.

WINDAUS, A. AND P. BORGEAUD. Ann. **460**: 235–239, 1928. Ueber die photochemische Dehydrierung des Ergosterins.

WINDAUS, A., P. BORGEAUD AND J. BRUNKEN. Nachr. Ges. Wissensch. Goettingen, Math.-physik. Klasse, 313–314, 1927. Die photochemische Oxydation und Dehydrierung des Ergosterins.

WINDAUS, A. AND J. BRUNKEN. Ann. **460**: 225–235, 1928. Ueber die photochemische Oxydation des Ergosterins.

WINDAUS, A., P. BUSSE AND G. WEIDLICH. Ztschr. physiol. Chem. **202**: 246–248, 1931. Nachtrag zu der Arbeit "Vergebliche Versuche zur Abtrennung des antirachitischen vom toxischen Faktor in Bestrahlungsprodukten des Ergosterins."

WINDAUS, A., K. DITHMAR AND E. FERNHOLZ. Ann. **493**: 259–271, 1932. Ueber das Lumisterin.

WINDAUS, A., J. GAEDE, J. KÖSER AND G. STEIN. Ann. **483**: 17–30, 1930. Ueber einige krystallisierte Bestrahlungsprodukte aus Ergosterin und Dehydro-ergosterin.

WINDAUS, A. AND A. HAUTH. Ber. **39**: 4378–4384, 1906. Ueber Stigmasterin, ein neues Phytosterin aus Calabar-Bohnen.

WINDAUS, A. AND A. HESS. Nachr. Ges. Wissensch. Goettingen, Math.-physik. Klasse, 175–184, 1927. Sterine und antirachitisches Vitamin.

WINDAUS, A. AND R. LANGER. Ann. **508**: 105–114, 1933. Ueber das 22-Dihydroergosterin.

WINDAUS, A., O. LINSERT, A. LÜTTRINGHAUS AND G. WEIDLICH. Ann. **492**: 226–241, 1932. Ueber das krystallisierte Vitamin $D_2$.

WINDAUS, A. AND A. LÜTTRINGHAUS. Ztschr. physiol. Chem. **203**: 70–75, 1931. Einige Bemerkungen über das antirachitische Vitamin aus bestrahltem Ergosterin.

Nachr. Ges. Wissensch. Goettingen, Math.-physik. Klasse, 4–7, 1932. Ueber die Zahl der Kohlenstoffatome in Molekül der Sterine, vor allem des Ergosterins.

WINDAUS, A., A. LÜTTRINGHAUS AND P. BUSSE. Nachr. Ges. Wissensch. Goettingen, Math.-physik. Klasse, 150–159, 1932. Neue Beobachtungen an Bestrahlungsprodukten des Ergosterins.

WINDAUS, A., A. LÜTTRINGHAUS AND M. DEPPE. Ann. **489**: 252–269, 1931. Ueber das krystallisierte Vitamin $D_1$.

WINDAUS, A. AND K. NEUKIRCHEN. Ber. **52**: 1915–1919, 1919. Die Umwandlung des Cholesterins in Cholansäure.

WINDAUS, A. AND O. RYGH. Nachr. Ges. Wissensch. Goettingen, Math.-physik. Klasse, 202–216, 1928. Ueber die Ester des Ergosterins und ihr Verhalten bei der Ultraviolettbestrahlung.

WINDAUS, A. AND R. TSCHESCHE. Ztschr. physiol. Chem. **190**: 51–61, 1930. Ueber das sogenannte "Isocholesterin" des Wollfettes.

WINDAUS, A. AND C. UIBRIG. Ber. **48**: 857–863, 1915. Ueber Koprosterin. (xxi. Mitteilung zur Kenntnis des Cholesterins.)

WINDAUS, A., F. v. WERDER AND B. GSCHAIDER. Ber. **65**: 1006–1009, 1932. Ueber die Zahl der Kohlenstoffatome im Molekül des Sitosterins und einiger anderer Sterine.

WINDAUS, A., F. v. WERDER AND A. LÜTTRINGHAUS. Ann. **499**: 188–200, 1932. Ueber das Tachysterin.

WINDAUS, A., K. WESTPHAL, F. v. WERDER AND O. RYGH. Nachr. Ges. Wissensch. Goettingen, Math.-physik. Klasse, 45–59, 1929. Einige Beobachtungen über die Ultraviolettbestrahlung des Ergosterins.

WISKOTT, A. Münch. med. Wchnschr. **75**: 1445–1448, 1928. Zur Vigantolbehandlung der Rachitis.

WISLICENUS, J. AND W. MOLDENHAUER. Ann. **146**: 175–180, 1868. Ueber das Cholesterindibromür.

WOOD, H. De puris natura atque formatione. Dissertation, Berlin, 45 pp. 1837. See p. 15 *et seq.*

WOODROW, J. W. Phil. Mag., series 7, **5**: 944–946, 1928. The ultra-violet absorption spectrum of cod-liver oil.

WYMAN, E. T. AND A. M. BUTLER. Am. J. Dis. Children, **43**: 1509–1518, 1932. Antirachitic value of milk from cows fed irradiated yeast.

YODER, L. J. Biol. Chem. **74**: 321–329, 1927. Effect of antirachitic vitamin on the phosphorus, calcium, and pH in the intestinal tract.

ZELLNER, J. Chemie der höheren Pilze. 257 pp. Engelmann, Leipzig. 1907. See pp. 27–39.

ZUCKER, T. F. AND M. J. MATZNER. Proc. Soc. Exp. Biol. Med. **21**: 186–187, 1924. On the pharmacological action of the anti-rachitic active principle of cod liver oil.

ZUCKER, T. F., A. M. PAPPENHEIMER AND M. BARNETT. Proc. Soc. Exp. Biol. Med. **19**: 167–169, 1922. Observations on cod-liver oil and rickets.

# THE NERVOUS CONTROL OF THE BLOOD VESSELS

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The investigation of the vasomotor system really began with the work of Claude Bernard (1851) who, following on the work of his fellow countrymen, demonstrated that section of the cervical sympathetic caused dilatation of the vessels of the ear of the rabbit and that stimulation of the peripheral cut end of the nerve caused constriction. In the latter observation Bernard was actually forestalled by Brown-Sequard (1852). Similar conclusions were arrived at by Waller (1853). It is interesting to note that Bernard was most impressed by the increased temperature of the rabbit's ear and thought that the nerve primarily controlled local heat production.

A further observation by Schiff (1856) suggested the existence of vasodilator nerves. He found that if an animal in which the section had been made on one side was exercised, the ear of the healthy side became warmer than that deprived of its vasoconstrictor supply. In 1858 Bernard demonstrated the existence of vasodilator nerves by finding an increased blood flow through the submaxillary gland, when the chorda tympani is stimulated. He also observed that the venous blood became bright red. Since that time an enormous amount of research has been carried out on the vasomotor system.

The mechanisms which control the vessels are by no means so clear-cut as those which control the heart. Two distinct influences constantly affect the blood vessels, the vasoconstrictor and the vasodilator mechanisms, which are each partly nervous and partly chemical. The impulses concerned emanate from the central nervous system, especially from the medulla where vasoconstrictor and vasodilator centres have been described, although, as in the case of the cardiac centres, these may really be the apices of reflex arcs. It might at first sight be reasonable to imagine that the vasoconstrictor fibres correspond to the sympathetic fibres which accelerate the heart and the vasodilator fibres to the vagus which slows the heart. This is, however, not true as a general statement, although it may be true of certain blood vessels. So far as present

knowledge goes, it may be considered that the sympathetic constricts especially the vessels of the skin and the intestines while it dilates usually but not always the vessels of the muscles and the heart. The parasympathetic appears to have a general dilator action, but it constricts the vessels of the heart. In exercise the sympathetic predominates.

In most work the term vasomotor centre is used to refer to the vasoconstrictor centre, but since the discovery of the vasodilator centres it seems best to use the second term.

THE VASOCONSTRICTOR CENTRE. The conception of special centres in the brain controlling blood vessels was first suggested by Schiff (1855) on the basis of the effect of section of the spinal cord. Nasse, for example, had observed that section of the cord caused an increased temperature of the hind limbs. The discovery of the centre, however, we owe to the workers in Ludwig's laboratory. Their starting point was the discovery that even after all the brain above the medulla was removed they still obtained a rise of blood pressure when they stimulated the central end of the sciatic nerve (Dittmar, 1870). This has been confirmed by Heidenhain (1870), Berkovitch (1875), Latchenberger and Deahna (1876), Asher and Lüscher (1889) and many others; Owsjannikoff (1871) subsequently studied the problem more minutely by progressive slicing of the brain from above downwards until the maximum fall of blood pressure was attained. The latter found also that the centre was bilateral and corresponded in structure to a group of cells known as the antero-lateral nucleus of Clarke and described by Dean and Koelliker as the lower diffuse part of the upper olive.

In the rabbit Ludwig and Owsjannikoff located a centre to a region 3 or 4 mm. long commencing 1 or 2 mm. below the corpora quadrigemina and ending 4 mm. above the tip of the calamus scriptorius, but this was further elaborated by Dittmar who described the upper limit as in the neighbourhood of the anterior fovea near the edge of the corpus trapezoides.

In 1916 Ranson and Billingsley re-investigated the problem and examined with weak electrical stimuli the floor of the fourth ventricle in cats, by the unipolar method. They found two points one of which gives a definite rise of blood pressure and the other a fall. The pressor point was on the lateral lip of the ala cinerea. The authors, however, realized that it may be that the points are really afferent pathways in connection with the centre, but their very localised position and response to stimuli which have no effect when applied to other regions suggest that they are definite centres. It may be, on the other hand, that they are points of reflex arcs which come near the surface.

Bikeles and Zbyszewski (1917) observed contractions of the vessels of the frog's web on stimulation of the medulla. If the muscular movements are excluded, however, it is stated that the contractions do not occur (Kolm and Pick, 1920).

The site of the centres has also been studied by Scott and Roberts (1923) and they have confirmed the above results in their essentials. Scott (1924) has compared the effects of cauterisation of the pressor and depressor points with the effect of the local application of strychnine. The effect of strychnine he found went apparently deeper than the cauterisation for, after the latter, a fall from stimulation of a somatic nerve may still be obtained but not after the former.

A similar separation of the activities of the vasomotor centres was found possible by Langley (1912), who found that if starch were injected into the internal carotid the effect of stimulation of the vagus and the internal saphenous could be prevented separately. It is, of course, possible that some of the effects of stimulation of a somatic nerve may take place through spinal arcs.

It has been suggested by Porter (1915) that there is a vasotonic as distinct from a vasoconstrictor centre. This is based on the fact that, after the injection of a sufficient quantity of alcohol, pressor reflexes are abolished while the height of the blood pressure is unchanged (Porter and Turner, 1915). This conclusion is quite erroneous. The results are really due to the fact that, as a result of the profound dilatation of vessels shown by the fall of venous pressure, the vasomotor centre is already fully stimulated as shown by the fact that section of the vagi causes a fall of blood pressure (McDowall, 1925). The reverse action is stated to occur with curari, i.e., an increase of reflex action is associated with the same tone. This I have been unable to confirm, but there are so many varieties of curari that I may not have used similar material.

*Spinal centres.* It was first shown by Goltz (1864) that even after the medulla is severed from the spinal cord, the vascular tone in the frog might recover, but that it was lost if the cord were destroyed. In the dog also he demonstrated that if the cord in the upper lumbar region were cut the temperature of the lower limbs became higher than that of the upper because of dilatation of vessels. Gradually, however, the vessels became normal again, but again they dilated if the lumbar cord were destroyed. Vulpian (1875) found also that, after section of the cord in the mid-dorsal region, constriction of one hind limb could be brought about by stimulation of the other and it is well known that stimulation of the central end of the sciatic nerve will cause a rise of blood pressure in a spinal animal.

This result is obtained even an hour or two after the cord is cut provided a small dose of strychnine is injected, but normally a week or two must elapse before such a result can be obtained without strychnine. This action of strychnine which may either reduce the synaptic resistance or increase the excitability of the cells, is discussed by Langley (1919).

All these experiments suggest the existence of subsidiary vasomotor centres in the spinal cord or at least that vasomotor reflex arcs occur in the cord. Further evidence supporting this view is that after the cervical cord has been cut section of the splanchnic nerves may cause a further fall of blood pressure.

The existence of these spinal centres has been further supported by the later experiments of Mathison (1910). According to Yates (1921) after section of the spinal cord in cats at different levels below the first thoracic segment the blood pressure recovers to a degree which depends upon the position of the section and the length of time allowed for recovery. The sympathetic outflow, kept under the control of the vasoconstrictor centre in the bulb, that is the segment in the bulbar side of the section, appears to be responsible for this recovery. She considers that the spinal cells of origin of the vasoconstrictor fibres below the transection do not take an important part, and that there are really no spinal centres of coördinate rank with the bulbar centres.

Similar experiments have been carried out by Bowen, Coombs and Pike (1922). They transected the cord aseptically in cats at varying levels between the 2nd and 9th thoracic. After recovery the animal was again anesthetised and it was found that removal of the cord below the section caused a further fall of blood pressure. They found, however, that whatever the state of the spinal cord interference with the medulla or upper part of the thoracic cord was always followed by a profound fall of blood pressure.

The evidence that the spinal centres normally play an important part is not very strong. They are, however, apparently responsive to the gaseous content of the blood (see the effect of carbon dioxide on the centres).

Some attempt has been made to localise the centres which have been found to lie in the dorsal (Vulpian, Kabierski, 1877) and in the lumbosacral region (Goltz, 1864, 1875). Since after section below the medulla further section does not materially affect the blood pressure, Stricker concluded that the centres were absent or scanty in the cervical cord; It was, however, noted by Dale and Evans (1922) that in the case

of the spinal animal, the preparation had a higher blood pressure if the cord were cut at the third cervical level than at the first.

That the sympathetic chain is also important is indicated by the experiments of Boshamer (1925) who found that division of the post-ganglionic fibres within half an hour of cutting the related pre-ganglionic fibres does not result in further dilatation. Increased dilatation occurs, however, if the post-ganglionic fibres are not divided until the following day.

*The relation of carbon dioxide to the vasomotor centre.* The first experiments specifically directed towards the study of carbon dioxide upon the circulation appears to have been carried out by Traube (1864) using mixtures containing as much as 20 to 75 per cent of the gas. He showed that it had a material effect on the circulation, which he considered to be due to the stimulation of the musculo-motor system. A little later Thiry (1864) in view of Traube's work came to the conclusion that the rise of blood pressure produced by the inhalation of carbon dioxide was due to stimulation of the centre causing a constriction of the peripheral arterioles. This was later confirmed by Traube (1864, 1865) who considered that carbon dioxide was an excitant of a vasomotor centre. Up to this time carbon dioxide had been generally looked upon as a harmful waste product. This stimulative action of carbon dioxide upon the vasoconstrictor centre was well recognised by Hill and Flack (1908), Plavec (1900) and by Mathison (1911). Hooker, Wilson and Connett (1917) in perfusion experiments of the medulla had also remarked on the peculiar efficacy of carbon dioxide as a general stimulant of medullary function.

It is, however, to Yandell Henderson (1907 et seq.) that we owe the conception of the necessity of carbon dioxide for normal vascular tone. With his associates he demonstrated that in an etherised animal or man a loss of carbon dioxide produced by over-ventilation of the lungs caused a profound fall of blood pressure and a variety of shock which was relieved by the administration of carbon dioxide.

It was not, however, until 1922 that Dale and Evans showed that the carbon dioxide is necessary for the normal function of the vasoconstrictor centre. Henderson's original explanation based on the finding of a low venous pressure and cardiac output was that the carbon dioxide was necessary for the normal relaxation of the venules. The loss of carbon dioxide was considered to allow the venules to constrict and dam up the blood (Henderson, 1917; Henderson and Harvey, 1918). This ingenious explanation, however, is liable to many objections, particularly the fact

that it does not explain the fact, pointed out by Dale and Evans (1922) that in an acute spinal animal over-ventilation causes a rise of blood pressure. Whether or not carbon dioxide is necessary for the activity of the spinal centres once they have taken over control in a spinal animal has not yet been investigated.

Dale and Evans (1922) showed that, while over-ventilation with air caused a fall of pressure in an etherised animal, over-ventilation with air containing carbon dioxide as present in expired air did not. They showed too that merely alkalinisation of the animal did not have the same effect as loss of carbon dioxide. The carbon dioxide may be considered to have a specific effect on the centre, no doubt because of its greater power than other acids of penetrating cell membranes; other acids stimulate the centre to a less extent.

It has been pointed out by Petroff (1931) that the pressure rise which occurs with asphyxia only occurs if the respiratory centre is in action, and it is suggested that the effects are really due to irradiation from the respiratory centre. This work requires repetition in view of the known effect of $CO_2$ on spinal centres. The rise does not depend on the secretion of adrenaline since the constriction is obtained in a separately perfused innervated hind limb (Tournade and Malmejac, 1933; McDowall, 1933).

According to Berthier, Deschamps and Halfern (1933) the injection subcutaneously of carbon dioxide causes a slow rise of blood pressure while the injection of air does not. This they consider to be due to an effect on the centre, but this, in view of the small amount injected, seems doubtful.

Later McDowall (1930) has shown that while some animals lightly anesthetised with chloralose may spontaneously recover from the fall of blood pressure which occurs if placed in the vertical (head up) position, they will not do so if they have been previously over-ventilated. They regain this capability of recovery if carbon dioxide is administered. In this connection it should be noticed that although it may be shown that in a chloralosed animal the normal postural reaction of the circulation requires carbon dioxide, the overventilation does not necessarily cause a fall of blood pressure because of the compensations which occur as in man. McDowall gives evidence that the constriction of peripheral capillaries is a result of the loss of a normal vasodilator action by carbon dioxide on these vessels. In this conclusion the work of Fleisch (1918, 1921, 1931) and of Atzler (1922, 1923) is supported. Fleisch particularly has emphasised the importance of the peripheral action of carbon

dioxide. The administration of ether to such an animal at once causes a failure to compensate and a fall of blood pressure, presumably because this drug paralyses the vessels which constrict just as it does the vessels, especially those of the skin, which constrict to minute doses of adrenaline (Dunlop, 1929).

It seems unlikely that the carotid and aortic reflexes and the subsequent secretion of adrenaline are concerned for Vercanteren (1931–32) has shown that they also depend on carbon dioxide and tend to be abolished by over-ventilation, but whether this is reliable evidence is somewhat uncertain for any fall of blood pressure by removal of the normal depressor stimulation tends to abolish the pressor effect of occluding the common carotid arteries or cutting the aortic nerves. Heymans and Bouckaert (1932) have however found that the effect of over-ventilation is greater than normal in animals in which the aortic and carotid nerves are cut.

In man, over-ventilation, not too violent but sufficient to cause a prolonged apnea, does not necessarily cause a fall of blood pressure. Collier, Densham and Wells (1927) examined 43 students and found the pressure fell more than 5 mm. in only 10. They suggest that in man the compensatory peripheral vaso-constriction is produced by the effort of the over-ventilation and loss of peripheral carbon dioxide. This view is supported by Norlin (1932). The occurrence of the peripheral constriction has been found by Boothby (1913) using the plethysmograph and by G. N. Stewart (1911) who found that if the hand was placed in a calorimeter less heat was given off during over-ventilation. At the same time it must be remembered that if the blood pressure is taken during very severe hyperventilation in man the blood pressure may be found to have fallen as a result of interference with the blood flow through the lungs as in Valsalva's experiment (Vincent and Thompson, 1928) but this fall is recovered from long before the apnea is at an end. Some persons show a great difference in the effect of over-ventilation which is greatly enhanced by the erect posture especially after influenza when the vasomotor reaction is weakened (L. Hill, 1895) or after a hot bath (Collier, Densham and Wells, 1927).

The failure of the blood pressure to fall may be ascribed to two causes: 1, to the mental effort involved in carrying out the over-ventilation (Collier, Densham and Wells, 1927) and 2, the peripheral washing out of carbon dioxide as the experiments of Dale and Evans (1922) in the spinal animal and those of McDowall (1930) on the compensating chloralosed animal suggest. That the skin vessels are concerned is

seen by the intense pallor which is seen in some persons on over-ventilation.

The reverse experiment can also be carried out, and it can be shown that the blood pressure of man does not rise materially when carbon dioxide is inhaled.* In some chloralosed cats also the inhalation is without effect but it may be shown by studying a perfused limb, still innervated, that the vasomotor centre like the respiratory centre has been stimulated. Presumably the dilator action of carbon dioxide compensates for the central constriction (McDowall, 1930c, 1933).

If, however, the compensation is poor or an anesthetic like ether is used, the administration of carbon dioxide causes a rise of blood pressure which is associated with constriction of the vessels of the intestine (Zuntz, 1878) and of the limbs.

For completeness it may be noted that the feeling of giddiness which may be produced by over-ventilation is present in those persons in whom the blood pressure does not fall. It is probably due to the fact that a reduction of the carbon dioxide of the blood reduces the dissociation of oxyhaemoglobin.

*The effect of oxygen want.* The effect of oxygen want on the centres has also been extensively studied, but for the most part in relation to the circulation as a whole, as the oxygen want affects not only the centres but the heart and vessels also. The effect of complete cerebral anemia produced by clamping the vertebral arteries which can be conveniently done by a special clamp (McDowall, 1930b) and by occluding the carotids is dramatic and is usually attributed to oxygen want. Previous over-ventilation of the animal, however, to reduce the carbon dioxide content of the blood so markedly delays the effect that there can be little doubt that the accumulation of carbon dioxide is much the more important agent in producing the effect (McDowall, 1933). It can also be shown that sudden hemorrhage causes intense constriction of the spleen and hind limbs of the animal perfused separately but in nervous connection with the body (Pilcher and Sollman, 1914; McDowall, 1933). The experiments of Mares (1902) and Mathison (1911) who administered nitrogen to animals show however a rise of blood pressure which can only be accounted for as due to oxygen want. According to Petroff (1931) the response to anemia persists after the response to carbon dioxide. He suggests also that the vasodilator centre is stimulated. This is very probable but some of his results may be accounted for by the anesthetic effects of large doses of carbon dioxide. Stewart, Guthrie, Burns, and Pike (1907) carried out a large number of experi-

*This is constant in part due to the activity of the depressor reflexes.

ments on the order of recovery of the various medullary centres after prolonged anemia. They have found that the vasomotor centre is one of the first to recover and later Stewart and Pike (1907 and seq.) found that the pressor responses to afferent stimuli were obtainable before the depressor effects. They found that in some instances the blood pressure recovered before the responses to afferent stimuli, a fact which they conclude indicates that vasomotor activity is independent of such stimuli.

From an investigation of the responses of the centres Gasser and Loevenhart (1913–1914) have concluded that oxygen want is of itself a stimulus and have put forward a theory to account for the stimulation.

*The spinal centres* are likewise responsive to the gaseous content of the blood: Cathcart and Clark (1915), Seppa (1919), Kowalewsky and Adamuk (1868), Sherrington (1909), Mathison (1910), Schlesinger (1874), Luchsinger (1878), Asher and Luescher (1899), Konow and Stenbeck (1889). The last named failed to get a rise of blood pressure with asphyxia when the cord was destroyed while Kaya and Starling (1909) pointed out that the spinal centres were sensitive to carbon dioxide only when there was also oxygen want.

For normal activity of all the centres a minimum of about 60 per cent blood pressure is necessary (Porter and Marks, 1908), a fact which presumably depends on oxygen supply.

THE EFFERENT VASOCONSTRICTOR PATHWAY. The efferent pathway has been studied by cutting or stimulating it at various points. Bernard (1862) had observed the fall of blood pressure caused by cutting the cervical cord while von Bezold (1863) found that if he stimulated the cut end of the cervical cord he could raise the blood pressure to seven times that before the stimulation. Ludwig and Thiry (1864) showed later that the effect did not depend on the cardiac nerves and pointed out the great part played by the splanchnic nerves in the reaction.

In attempting to localise the spinal pathways more accurately, Nicolaides (1882) found that the pathways were partly crossed but chiefly uncrossed for he found that if the cord were divided longitudinally, stimulation of the right side caused the right kidney to contract more than the left. The later experiments of Ranson and Billingsley (1916) suggest that the constrictor impulses pass down the antero-lateral columns.

The elucidation of the pathway beyond the spinal cord we owe especially to Gaskell, Anderson, and Langley, who identified the efferent constrictor fibres with the thoracicolumbar outflow.

A number of early workers had described the sympathetic as taking origin from all the spinal nerves. The idea that the white rami were absent from the upper and lower regions of the cord was first put forward by Beck (1846) on the basis of anatomical dissections on man, but his observations generally passed unnoticed. Reissner (1862) described bundles of small nerve fibers as being peculiar to the anterior roots of the dorsal region of the cord. The idea that the cervical sympathetic arose from the lower cervical nerves which had become generally accepted was, however, finally dispelled by Bernard (1862), and especially by Langley and Gaskell.

It is to Gaskell that we owe the elucidation of the problem generally although more modern research may modify his views slightly.

In 1883 Gaskell suggested that the vasomotor (meaning the vasoconstrictor) fibres took origin from the lateral horn of the spinal cord and this was later confirmed by Anderson (quoted by Gaskell, 1916) who found that section of the cervical sympathetic in kittens caused a failure of growth of the cells of the lateral horn.

Gaskell (1886) approached the subject from the histological and morphological standpoint.

A number of earlier workers, such as Bidder and Volkman, had shown that the white rami consisted mainly of small medullated fibres. Gaskell (1886) found in the dog that bundles of small nerve fibres 1.8 to 3.6$\mu$ in diameter occurred in the anterior roots of those which had white rami but not in the roots with grey rami except in the case of the origin of the sacral nerves which give rise to the nervus erigens. This observation is not quite accurate for subsequently Langley (1896) found that the grey rami of the cat and some other animals do contain a considerable number of small medullated fibres but Langley, on the basis of degeneration experiments (1921) has concluded that these fibres arise from white rami or are afferent fibres. The effect of stimulation of the spinal nerves at their origin confirmed this view.

The effect of section of the efferent pathway depends on the point of section. The greater the area affected the greater is the effect on the blood pressure. Section of the cervical cord has therefore the maximum effect and, of individual nerves, section of the splanchnics causes the greatest lowering of blood pressure. The lowering is brought about not only by the diminution of the peripheral resistance but also by the greatly increased capacity of the vessels concerned. The effect of section of the splanchnic is stated to be greatest in amount in animals with a relatively large amount of gut. It should be emphasised, too, that

the effects of the splanchnic area vary as is illustrated well by the experiments of Rutherford (1869) who found that section of the vagi (and aortic depressor) caused a rise of blood pressure in a digesting but not in a fasting dog. The fall of pressure produced by section is much more marked in the dog than in the cat, but even then there may be complete recovery after 11 or 12 days (Asp, 1867; Biasotti, 1927). The effect produced must also depend very materially on the extent to which the aortic and carotid nerves are active and to demonstrate a fall on section of the splanchnics it is necessary to put these nerves out of action (Kremer and Wright, 1932). According to Burton-Opitz (1917) section of the splanchnic doubles the blood flow in the areas concerned, while, according to Herrick, Essex and Baldes (1932, 1933), removal of the lumbar sympathetic doubles the blood flow in the femoral artery on the operated side as measured by Rein's thermostromuhr. This effect is permanent.

The pathway of the vaso-constrictor nerves after they leave the sympathetic trunks is of considerable interest, as it has become common to cut the sympathetic paths to a limb in order to improve its blood supply.

We believe that normally the majority of the fibres leave the sympathetic by the grey rami to the spinal nerves and are distributed with the ordinary somatic nerves. In the head and neck and in the abdomen it appears certain that instead of or as well as being distributed by somatic nerves, the sympathetic nerves travel on the arterial trunks. Where in the case of the extremities the peripheral nerves take over the constrictor distribution is difficult to decide; it may be that the vessels get a series of supplies from peripheral nerves. General anatomical opinion, however, inclines to the view that the change-over occurs near the root of the limbs. It has been stated by Stahl (1924) that section of the periarterial plexus has the same effect as section of the whole sympathetic trunk, but it is difficult to see how these results can be correct in view of the fact demonstrated by anatomists that the arterial plexuses receive branches from nerves at several places. (See "Capillaries.") There is however an increasing volume of evidence that such nerves are afferent rather than efferent.

In this connection it is of interest that when an artery is obliterated during life by thrombosis, the nerves are not necessarily destroyed. According to Lancelin (1917) division of such an artery has the same results as division of vaso-constrictor nerves, but the later work of Leriche suggests that an embolus may interfere with the nerve supply.

A vast amount of work has been done on the effects of stimulation of the peripheral pathway at different points; indeed, such work has been largely the basis of our knowledge of the anatomy of the autonomic nerves generally and is chiefly of anatomical and surgical interest.

By stimulation and injecting bromphenol blue (3 cc. of a 4 per cent solution) Gilding (1932) has been able to study the distribution of vaso-constrictor nerves at the periphery. The vaso-constriction is evident by the absence of staining in the areas affected. In this way he found that the sympathetic nerves follow the motor or sensory nerves to the periphery branching from them in the vicinity of the small vessels supplied. Stimulation of the stellate ganglion causes vaso-constriction in skin, mucous membrane and muscles, bone-marrow, meninges, choroid plexus and thyroid. In spite of arterial anastomosis the vaso-constrictor impulses appear to stop short at the middle line.

*The chemical tone of vessels.* Even after all the nerves to a part are cut, the vessels eventually recover their normal calibre as first pointed out by Goltz and Ewald (1864) and by Goltz and Freusberg (1874). They removed the lower part of the spinal cord of a dog and found that the hind limbs which were at first hotter became as cold or cooler than the normal forelimbs. This experiment has been amply confirmed (Sherrington, 1906; Pike, 1908, 1909, 1912; Porter and Mulberg, 1900). Complete cerebral anemia produces a rise, then a prolonged fall, but eventually there is recovery. Goltz and Freusberg (1874) obtained similar results in dogs after cutting a sciatic nerve. Sometimes after a few weeks the paralysed paw had a lower temperature than the normal.

Goltz, Freusberg, and Gergens (1875) in later experiments still further emphasised the existence of residual tone which did not disappear on cutting the nerve by demonstrating that even after the nerve was cut, mechanical stimulation of the nerve such as by cutting would cause a peripheral dilatation.

The return to normal of the blood pressure after removal of the sympathetic chain in the experiments of Stahl (1924), of Cannon (1931) and of Bacq, Brouha and Heymans (1932 and 1933) shows that a tone exists which does not depend on the sympathetic.

Removal of the right lumbar sympathetic chain and severance of the right splanchnic causes an initial fall of blood pressure followed by a prompt rise reaching a level well above the pre-operation level but gradually returning to normal afterwards. If the remainder of the chain be now removed, the blood pressure drops lower than previously and returns to normal. Biasotti (1917) also removed the sympathetic

chain from the 12th thoracic to the 4th lumbar together with section of both splanchnics and produced similarly only a slight and temporary fall of blood pressure in nine dogs. Stahl (1924) compared also the effect of cutting the peri-arterial plexus and found that it was the same in character as severance of the sympathetic trunk but different in degree. Sharpey Schafer (1919) and v. Brücke (1931) also found that after section of the cervical sympathetic there is recovery of the tonus of the vessels of the ear.

In considering the return to normal, Yates (1921) concluded that the spinal cells are little concerned, if at all. In regard to the vessels responsible for recovery, the work of Herrick, Essex and Baldes (1933) who measured the blood flow in the femoral artery thirty-four months after lumbar sympathectomy and found that an increased flow in the operated side is maintained after that period indicates that the large vessels are not concerned in the recovery. We may, therefore, look to the capillaries and this seems most probable on general grounds. This is supported by the observations of Tournade and Malmejac (1933a, 1933b) who stimulated the nervus erigens—that is, the dilator nerve of the penis —with the vaso-constrictor supply cut. They found that later a peripheral vasoconstrictor mechanism came into play to antagonise the vasodilatation.

The exact cause of this return to normal is a matter of considerable discussion. Krogh (1929) suggests that capillary tone at least is maintained by the circulation of the vaso-active principle of the pituitary body, since in the frog removal of the pituitary body causes capillary dilatation while an extract causes them to constrict if perfused in very dilute solution. On the other hand it is well known that removal of the pituitary body does not cause generalised vasodilatation in mammals and the evidence that the pressor substance dialysed from the blood by Krogh and his colleagues was actually pituitary extract is by no means secure. In perfusion experiments it is possible to maintain the tone of capillaries in different ways. Dale and Richards (1918) did so by adding adrenaline to the perfusion fluid while Hemingway and McDowall (1926) pointed out that if steps are taken to prevent the accumulation of acid products in the perfused tissues it is possible to maintain a vascular tone, dilatable by histamine, by perfusion with an alkaline Ringer's fluid. This view is supported by the earlier work of Fleisch (1918) whose experiments were apparently misinterpreted by Krogh. Such experiments suggest that after the nerves have been cut, chemical agents normally circulating in the blood-stream can take over the control

of the vessels which become, at least temporarily, more sensitive to chemical influences after denervation as the experiments of Dale and Richards (1918) indicate. Burn (1925) has shown that when the nerves are fully degenerated the reaction disappears. What happens when the vessels have recovered their tone is not known. Of recent years the subject of recovery after denervation has been largely studied on man by surgeons who have sought to relieve vascular spasm, e.g., that of Raynaud's disease by removal of the sympathetic supply to the part. In some instances especially in the case of the upper limbs the preliminary dilatation of the vessels is succeeded after some months by a recovery of the vascular tone. In the lower limbs the dilatation is more permanent (Learmonth, private communication) and may be present after many years. Surgeons generally consider that the occasional recovery in the upper limbs is due to inadequate denervation, but it is a little difficult to imagine that there is a fundamental difference between the reactions of man and of the lower animals although the clinical evidence appears to point in this direction. It is important in all such work to realise that a recovery from spasm brought about through nervous paths does not necessarily mean that the vessels have not recovered their tone. Accurate temperature controls are essential but even an increased temperature of the part does not exclude capillary as distinct from arterial recovery. It is possible that some of the upper limbs' recovery may be the result of compensatory reduced vasodilator tone or as suggested by Leriche to some peripheral nervous mechanism as yet unknown. Reviews of the effects of ganglionectomy and sympathectomy have been published by Brown and Adson (1929) and by Gask and Ross (1934).

*The vessels innervated.* In all the work prior to 1914 it was generally assumed that the vasomotor mechanism primarily controlled the arterioles since these vessels are the most muscular of the vascular system. The work of Dale and Richards (1918) and of Dale and Laidlaw (1918) and of Krogh (1919, 1920, 1921) has since emphasised, however, the importance of the capacity of the capillary bed in the general control of the circulation.

The fact that capillaries have a nerve supply has been repeatedly shown since it was first demonstrated by Beale in this College in 1860 but it took many years for the physiological significance of this observation to be appreciated.

As it used to be thought that the peripheral resistance was chiefly located in the arterioles it has been assumed that vasomotor nerves

exerted their activity on these vessels, but the work especially of Dale and Richards (1918) and of Krogh, summarised in his monograph (1923, 1929), has drawn attention to the fact that not only do the capillaries change their calibre but also are responsible for part of the peripheral resistance and are a primary consideration in the capacity of the circulation. The nervous control of these vessels has therefore become of great interest and importance. The experiments of Hooker (1920) who observed the ear of the rabbit microscopically when the cervical sympathetic was being stimulated placed beyond doubt the fact that not only the arterioles but also the capillaries are caused to diminish in calibre; whether the shutting of the capillaries is active or passive is difficult to determine, but the former is most probable.

This has been confirmed on the ear of the rabbit even when all the blood flow has been arrested (Harris and Marvin, 1927). It has also long been known that the capillaries of the frog's web do not necessarily close when there is marked contraction of the arteries (Roy and Graham Brown, 1879; Krogh, 1920). Langley (1911) has, however, emphasised that often the capillaries do diminish in diameter when the pressure falls as a result of local constriction of arterioles. The exact conditions, especially those of temperature and pressure, of individual experiments no doubt profoundly affect the results.

For some unexplained reason Hooker (1920) failed to find any dilatation of the capillaries of the cat's ear when the cervical sympathetic was cut, but Rehberg and Krogh (1929) have observed such dilatation in a rabbit. Krogh also noted that the intense vascular (arteriolar and capillary) constriction which occurs in a rabbit breathing air with a diminishing amount of oxygen is abolished or greatly reduced by cutting the cervical sympathetic. In the rabbit the tone of capillaries as well as of the arterioles has been observed to return after a day or two but the capillary tone is said to be much less stable (Krogh, 1929). Breslauer (1919) made similar observations on patients with nerve lesions. Krogh (1920) has been quite unable to demonstrate constriction of the capillaries of the frog's tongue although arteriolar constriction was observed, but he points out (p. 82, 1st ed.) that the constriction which may be produced by a strong mechanical stimulus of an artery of the skin or web may spread to neighbouring capillaries.

In man the occurrence of capillary constriction due to nervous influence is very well proven. In 1879 Rouget considered that the pallor of the face in emotion could only be explained by capillary constriction. This has actually been observed using the skin microscope by Carrier

(1922). That blueness is produced in the fingers, tips of the ears, or old scars in cold weather is probably also due to arteriolar constriction without capillary constriction and may also be taken as evidence that the capillaries are not necessarily collapsed when the arterioles constrict. In 1920 Leriche and Policard reported an instance of contraction of the capillaries at the base of the nail in man when the adventitial coat of the brachial artery was stimulated mechanically.

It may then be taken that generally the vaso-constrictors affect both arterioles and capillaries. Difficulty, however, arises in relation to the muscles and lungs where the exact action of the vaso-constrictors is not yet clear, and adrenaline may be shown to cause both constriction and dilatation of vessels.

The question of the pathway to the capillaries has been raised by Wiedhopf (1923) who found that in plethysmographic studies of the dog's foot removal of the fibres on the wall of the femoral artery produced no change and reactions to painful stimuli is still present. Such experiments suggest that the supply to the foot is given off to the vessels below this level, but it is not possible to discuss in this review points of more purely anatomical detail.

*The veins.* The fact that veins are capable of constriction may be observed by anyone who attempts to insert a cannula into one without due care. Stimulation of the sciatic or anterior crural has been found to cause constriction of the superficial veins of the hind leg of a dog (Pal, 1888; Thompson, 1893; Cavazzani and Manca, 1895; Bancroft, 1898; Ducceschi, 1902) while Mall (1890) and later Bayliss and Starling (1894) and also Schmidt (1909) and Burton Opitz (1913) showed that the branches of the portal vein could be caused to constrict. Henderson (1917) has described constriction of the mesenteric veins when the intestine is injured. This may be nervous or due to the liberation of chemical constrictors, probably to both. Hooker (1918b) also obtained evidence of constriction of veins of the large intestine on stimulation of the nerve fibres from the inferior mesenteric ganglion and in 1921 Donegan, using a method similar to that of Hooker (1918b) found that the veins of the hind limbs of a cat and dog receive sympathetic fibres which originate in the second, third, and fourth lumbar roots. He also (1918b) studied the mesenteric veins connecting it with a manometer, the artery being fully open. He obtained constrictions from stimulation of the nerve from the inferior mesenteric ganglion and less from the saphenous and from asphyxia. He concluded that the veins are under central control. The cutaneous veins of the hind limb receive both

constrictors and dilators from the sciatic and either may be demonstrated according to the nature of the stimulus used (Horiuchi, 1924).

The old experiment of Goltz (1864) later confirmed by Tawastjerna (1916) indicated that the veins are in a state of tonus which may be abolished in the frog by tapping the intestine. The rise of venous pressure which Henderson, Barringer and Harvey (1909) found occurs if carbon dioxide is administered is probably due to an action of the vasomotor centre upon the veins and the general capacity of the circulation, although the authors themselves thought otherwise and believed it to be due to a relaxation of venules. This has already been discussed in relation to the effect of carbon dioxide on the vasomotor centre.

Fleisch (1931) has also demonstrated that the existence of venous tone in isolated veins connected with the nervous system can be inhibited or increased reflexly as a result of changes in pressure in the carotid sinus. The veins like the arteries appear to be under a tonic influence from the vaso-constrictor centre (Hooker, 1918b; Donegan, 1921), since they dilate when their nerves are cut.

A number of workers have demonstrated that the veins respond reflexly to changes in the carotid sinus (Heymans and Bouckaert, 1930; Heymans and Dautrebande, 1931; Gollwitzer-Meier and Schulte, 1931).

When the central end of the vagus in the cat (i.e., the aortic depressor fibres), is stimulated there is a very marked fall of venous pressure. This may be considered to be due to the capacity effect of the opening up of so many blood vessels but the investigations on the carotid sinus suggest also a direct action on the veins. This fall of venous pressure is not so easily obtained in the rabbit.

All these experiments are, however, important as they suggest the possibility of the existence of a large venous reservoir, the capacity of which may be changed and produce enormous capacity effects on the circulation. How far the venous system is really important is very difficult to assess, for the venous pressure can readily be shown to fall after hemorrhage which is adequately compensated for on the arterial side. If the capacity of the venous reservoir were readily adjustable a compensation on the venous side would be expected also. How far this occurs does not appear to have been studied.

METHODS OF STIMULATING THE VASOMOTOR CENTRES. *The influence of the higher centres.* The fact that the blood pressure may rise during conditions of emotion was first described by L. Hill (1898). A rise of as much as 90 mm. of mercury has been recorded in a subject under the

emotion of being about to give an important lecture. The occurrence of cerebral hemorrhage during emotion may be considered also to be due to the same cause. So far no experiments on the effect of emotion after atropine to exclude cardiac effects appear to have been carried out. Sufficient direct observations have, however, been made to indicate that vaso-constriction of the skin vessels at least does occur under emotion. The pallor and sensation of cold produced by fright or the seeing of something disagreeable was long ago ascribed by Mosso to withdrawal of blood from the skin. This is all summarised by Mosso in his monograph (1923).

Direct observation with the skin microscope has shown constriction of the superficial capillaries during a thunderstorm (Carrier, 1922) and similar constriction of the vessels of the ear of a rabbit has also been observed if the animal is frightened, indeed care has to be taken to avoid this if it is intended to draw blood from an ear vein.

It must be admitted that in all these experiments no differentiation has been made between the action of sympathetic nerves and the effect of adrenaline secretion which occurs under similar circumstances. Hargis and Mann (1924) placed the spleen of a dog in an aseptic plethysmograph and, after healing had taken place, found that emotional states were specially liable to cause changes in spleen volume. The exteriorised spleen has also been observed to constrict from purely emotional causes (Barcroft and Stephens, 1927; Barcroft, 1929; Barcroft and Florey, 1929). The same observations have been made on the colon similarly made visible by fastening it outside the abdominal wall (Drury, Florey and Florey, 1929).

Of recent years attention has been drawn to the hypothalamus in relation to the circulation especially as a result of the work of Karpus and Kreidl (1927) who obtained a rise of blood pressure from stimulation of this region. While there is evidence from other sources that sympathetic centres may exist in this part of the brain, there is little doubt that the functions assigned to it have been multiplied unduly. Nevertheless the undoubted fact that the hypothalamus is responsible for the regulation of body temperature as shown by the loss of temperature control when the region is removed and by the peripheral effects of the application of heat and cold to it would lead one to expect vascular changes from stimulation. Presumably also it is possible to stimulate the descending pathways from the higher cerebral centres to the medullary centres. To assign any supreme function to the hypothalamus does not in the light of present knowledge seem any more justifiable than to assign

special functions to the pons, lesions of which are known to produce occasionally remarkable changes in body temperature and blood pressure.

*Stimulation of afferent nerves.* Stimulation with a strong rapidly alternating current of any afferent nerve except the aortic and carotid causes vaso-constriction as indicated by a general rise of blood pressure, cardiac effects, or the secretion of adrenaline being excluded. This was noted by many of the early observers. The methods actually used are conveniently shown in tabular form below. The rise is proportional to the strength of the stimulus (Dittmar, 1870) and Langley (1924) has pointed out that the effect is less in an eviscerated or hungry animal. The medullary centres need not, however, be present for this to occur for the rise is seen in a spinal animal (Dittmar, 1870); indeed it is often better seen in spinal animals in which the compensatory influences of the carotid and aortic nerves are absent. If these nerves are retained intact the best results are obtained in an animal which is narcotised with chloralose until the pupils are constricted but the corneal and ear reflexes still present. Curari reduces the responses (Dogiel, 1871). In all investigations it is most desirable not only to use different anesthetics but different degrees of anesthesia and to compare the results in decerebrate animals which however usually have the disadvantage of toxic absorption, carbon dioxide loss and also loss of the control of the vessels used for the regulation of body temperature. Moreover anesthetisation and operative procedures themselves exhaust the suprarenal glands and reduce pressor response, but the effects are recovered from—if adequate time is given—usually about one hour.

Since weak stimuli cause a fall of blood pressure, the exact strength of stimulus necessary to cause a rise of blood pressure has been studied by Martin with Lacey (1914), Stiles (1914), Mendenhall (1915), Jacoby (1922) and by Gruber (1917) who has concluded that whether or not a pressor or depressor effect is produced depends on the volume of impulses reaching the central nervous system. Support to this view is also given by the earlier work of Grützner and Heidenhain (1877) who in stimulating the skin emphasised the area stimulated rather than the intensity. It is suggested that the strength of stimulus necessary to produce a pressor response is so great that it may be considered unphysiological and that a fall of blood pressure must be considered the normal response of sensory stimulation which is short of pain. Martin and Mendenhall (1915) however point out that whether or not a weak stimulus causes vasoconstriction in the ear of unanesthetised rabbit depends on the psychic stimulation produced.

It might be expected that severe pain would cause a rise of blood pressure in man. Records on this subject are remarkably scanty, but Willway (unpublished) studying the effect of dressing painful wounds has found that, while a rise is usual, it is not nearly so marked as would be expected or as may occur with purely psychic stimuli. It may, however, be concluded that normally harmful stimuli have at the same time a marked psychic effect.

The matter is discussed further in a later section on the reciprocity of the centres.

The sensitivity of the centre to afferent stimuli is stated to be enhanced by concussion (Porter and Storey, 1907).

The pressor impulses from somatic nerves to the centre pass up the spinal cord by Lissauer's tract, a tract composed of many neurones (Ranson and Billingsley, 1916) but those which pass up from the splanchnic nerves pass up separately since division of the tract does not obliterate the pressor response (Davis, 1921).

It has been pointed out by Porter and Richardson (1908) that there is no difference in the rise produced on stimulation of a mixed nerve such as the sciatic in different animals, e.g., rabbit, cat, rat, hen, guinea pig, while Martin and Stiles (1914) have shown that if stimulation of one region ceases to be effective stimulation of another may be effective.

*Rise of blood pressure.* The various methods by which a reflex rise of blood pressure may be produced may conveniently be shown in tabular form. In most, however, cardiac and adrenaline effects have not been excluded.

| | |
|---|---|
| Central end of almost any afferent nerve | Magendi quoted by Vulpian (1875) v. Bezold (1863) |
| Posterior roots, splanchnic | Asp (1867), Mall (1880) Auer and Meltzer (1912) |
| Dura mater | Howell and Austin (1900) |
| Spinal cord | Gray (1912) |
| Sense nerves | Conty and Charpentier (1877) Dogiel (1880). Vincent and Thompson (1929) |
| Vagus, trigeminal | Lovén (1866) |
| Posterior auricular | Lovén (1866) |
| Trigeminal by nose | Allen (1928) |
| Thoracic sympathetic | Davis (1922) |
| Gastric | Berkowitsch (1875) Mayer and Pribram (1872) Ganz (1870) |
| Erigentes and hypogastric (the latter being the greater) | Courtade and Guyon (1901) |

| | |
|---|---|
| Radial, median ulnar, accelerans and others but obtained negative results from recurrent, accessory and phrenic | Grossman (1897) |
| Optic and facial | Martin and White (1922) |
| Pressure on eye | Ashner (1908) |
| Noise, depending on nature | Dogiel (1880) |
| Humane killer | Tweed, Clark and Eddington (1931) |
| Distention of stomach | Dimitrenko (1916) |
| Hunger pains | Carlson (1913) |
| Vagus, central end | Kowalewsky and Adamük (1868A)<br>Sollman and Pilcher (1912)<br>Roy and Adami (1892)<br>Aubert and Roever (1868)<br>Langley (1912)<br>Many others |
| Gastric vagus | Miller (1912) |
| Carotid nerve | Tournade and Malmejac (1930)<br>Danielopolu, Marcu, Proca and Aslam (1932) |
| Posterior roots, greater than spinal nerves | Bradford (1889) |
| Asphyxia | See special section |
| Carbon dioxide | See above |
| Oxygen want | See special section |
| Compression of forearm | Martin and White (1922) |
| Application of irritants to arteries | Heger (1887), Stephani (1895)<br>Kaufmann (1912), Pagano (1900),<br>Fleisch (1918) |

*Constrictor reflexes.* The reflex effects of afferent stimulation of the vasomotor centre have also been studied and the changes produced, specially in the blood flow, temperature, volume, or appearance of different parts of the body. These may be conveniently shown in tabular form.

| *Region affected* | *Stimulus* | |
|---|---|---|
| Kidney | All varieties of stimuli especially cold | Conheim and Roy (1883) |
| | Sciatic | Wertheimer (1894)<br>Burton Opitz and Lucas (1909) |
| | Central thoracic sympathetic | Burton Opitz (1917) |
| Kidney, spleen, and liver | Crural | François Franck and Hallion (1896) |
| | Vertebral | François Franck (1899) |
| Kidney and intestine | Nose | François Franck (1899) |
| | Sole and foot | Preoabschenesky (1892) |

| | | |
|---|---|---|
| Stomach and intestine | Brachial | Langley (1924) |
| Intestine | Sensory nerves generally | Bunch (1899) |
| Colon | Emotional stimuli | Drury, Florey and Florey (1929) |
| Kidney and spleen | Noise | Corbeille, Corbeille and Baldes (1930) |
| | Sciatic | Sollman and Pilcher (1910), (1912) |
| Spleen | Emotion | Benhamon, Marchioni and Nouchy (1929) |
| | Sensory nerves | Roy (1881) |
| | Emotion | Hargis and Mann (1924) |
| | Exercise | Barcroft and Stephens (1927) |
| | | Barcroft and Florey (1929) |
| Extremities i.e., skin | Sciatic and median | Bayliss (1908) |
| | | Bayliss and Bradford (1894) |
| | | Vulpian (1875) |
| Opposite side | | Maragliano and Lusona (1889) |
| | Dorsalis pedis | Edgren (1880) |
| Skin (but muscles dilated) | Temperature and various nerves | Heidenhain (1870) |
| | | Wertheimer (1894) |
| | | Azzi (1920) |
| Skin | Volition | Mosso (1923), Saski (1910) |
| | Stimulation of all higher senses | Hallion and Comte (1894) |
| Hind limbs | Shock produced by intestinal manipulation | Seelig and Lyon (1909) |
| Skin | All sensory stimuli | Patrizi (1897) |
| Muscles of thigh | Vagus | Gaskell (1878) |
| Ear | Pulling on intestine | Pavlov (1878) |
| Ear (occasionally) | Posterior auricular | Snellen (1858) |
| | | Lovén (1866) |
| Arterio-venous anastomosis | Cold | Grant and Bland (1931) |
| Ear | Depressor (aortic) | Dastre and Morat (1884) |
| | | Langley (1925) |
| | All sensory stimuli including vagus and trigeminal | Eckhard (1873) |
| Nose | All stimuli including heat and cold | Tschalussow (1913) |

| | | |
|---|---|---|
| Tongue | Sciatic. | Vulpian (1875) |
| Thymus | Crural | Hallion and Morel (1912) |
| Thyroid | Vagus | François Franck and Hallion (1908)<br>Sinakewitch (1906) |
| Frog's web | Ear, eye, skin | Pick (1872)<br>Stepanow (1918) |
| Arteries and veins of skin | Cold | Lefevre (1899), Hallion and Comte (1899)<br>Hough and Ballantyne (1899)<br>Pickering (1932) |
| Fingers, ears, nose cheek | | Lewis (1931) |
| Opposite hand | Cold to hand | Brown-Séquard and Tholozan (1858)<br>Stewart (1911)<br>Stewart and Walker (1913)<br>Densham and Wells (1927)<br>Stephani (negative results, 1895)<br>Sewall and Sandford (1890)<br>Stewart and Laffer (1913)<br>François Franck (1890) |
| Extremities but more blood to head and retina | Cold to foot | Müller (1905) |
| Constriction followed by dilatation | Cold to foot (probably psychological) | Müller (1905) |
| Respiratory passages | Cold to any part of body | Azzi (1920) |

It may in general be stated that stimulation of practically any part of the body results in vaso-constriction which may be generalised or local in its distribution. From the experiments quoted it may be concluded that any stimulus other than that of warmth may cause constriction especially of the skin and splanchnic region—an observation which is no doubt related to mobilising of the blood for muscular activity as suggested originally by Heidenhain. It is interesting to remark also that Patrizi (1897) and also Cavani (1903) found that the reflexes were more active on the right side in right-handed persons, while Grutzner and Heidenhain (1877) and also Lewaschew (1884) have pointed out that

vaso-constrictor nerves exert their greatest activity at the periphery of the limbs, i.e., where the proportion of skin is greatest. This fact may be related to the observation of Woollard (1926) that medullated nerves are most abundant on the peripheral vessels.

*The reciprocity of the centres in reflex vaso-constriction.* In all these experiments we can presume that the vasomotor centre has been stimulated. Bayliss (1908) demonstrated that the reaction occurred in the absence of the vaso-dilators by previously cutting the posterior roots and obtaining diminution in the volume of the hind limb of a cat on stimulation of the central end of the median nerve. This matter is discussed later.

*The cause of the tonic activity of the vaso-constrictor centres.* That the vaso-constrictor centre is constantly sending out impulses to the blood vessels is evident from the fact that separation of any part of the body so far investigated from this point of view from the centre results, as we have already seen, in a dilatation of the blood vessels in that region, and if the region is large enough, e.g., the splanchnic, to a fall of blood pressure. It should, however, be emphasised, as pointed out by Kremer and Wright (1932) that in order to show such tonic action it may be necessary to throw out of action the aortic and depressor nerves which tend to prevent the fall of pressure.

Whether or not the centres possess some inherent activity possibly dependent on the presence of carbon dioxide or whether the sending out of impulses is dependent on the receiving of efferent impulses is a difficult problem. If the centre is looked upon merely as the upper limit of a number of reflex arcs it is easy to see that carbon dioxide may simply act through causing facilitation in the arcs, for it has been shown that many of the activities of the brain are dependent on carbon dioxide. We have already referred to the effect of the loss of carbon dioxide on cerebration. It has also been shown that the postural reflexes are dependent on carbon dioxide (McDowall, 1930a).

The problem is very similar to that of the exact nature of the respiratory centre. At first sight the reflex conception appears reasonable but the only experiments on the isolated brain which were carried out by Adrian and Buytendijk (1931) on the brain of the gold fish suggest that the respiratory centre at least has an inherent rhythm as indeed have some of the cells connected with spinal reflexes. Much work is still required on this subject, but the difficulty is to obtain evidence of the activity of the centres in the absence of afferent impulses. It must be remembered in such experiments that the cut ends of nerves may conceivably give rise to stimuli.

Stewart and Pike (1907) in discussing the subject conclude that both the chemical and nervous stimuli normally play a part in maintaining the tone of the centre. Recent work by Rein (1930) indicates, however, that stimulation by carbon dioxide is distinct in its effects from reflex stimulation. The former causes a constriction but the latter produced by lowering the pressure in the carotid sinus causes a dilatation in muscle vessels. But Foa (1920) has concluded that afferent stimuli are unnecessary. Sollmann and Pilcher (1910) remarked that asphyxia was a much more efficient stimulus than sciatic stimulation. There is a certain amount of evidence that the tonic action of the centre is subject to rhythmical changes in man and animals. This may show itself in the rhythmical waves associated with the names of Traube (1865) and Hering (1869), Adrian, Bronk and Philips (1932).

Lewin and Schilf (1927) also have recorded that the rhythmical movements which are often observed in the vessels of the rabbit's ear cease if the vaso-constrictor pathway is cut, but how far this is general to the rhythmical movement seen in other vessels is uncertain for those described by McDowall (1921) were observed in perfusion experiments. Langley (1911) for example in the frog's web, discussing spontaneous movements, points out that although they appear to depend chiefly on the central nervous system, they may still occur in a region with degenerated nerves.

The general nutrition of the vessels appears to depend in some degree on the central vasomotor activity since, if a limb is denervated, marked degeneration, especially of the muscular tissue of the media occurs. The elastic tissue disappears (Kerper and Collier, 1927).

*Pressor impulses from the vascular system itself.* The fact that stimulation of the central end of the vagus may sometimes cause a rise of blood pressure has long been known and in 1879 it was pointed out by Pavlov that if the blood pressure was lowered by hemorrhage, section of the vagi caused a further fall of blood pressure. McDowall (1924) in studying the effect of alcohol on the circulation, found that after the injection of this drug intravenously although the arterial pressure was little affected the venous pressure fell markedly, and that if the vagi were cut the arterial pressure fell. He went on to show that any condition which caused a fall of venous pressure brought into operation a vago-pressor reflex. The existence of such a pressor reflex by way of the vagus was subsequently confirmed by Anrep and Starling (1925) in crossed circulation experiments, and by Anrep and Segall (1926). Reed (1925) has also confirmed the existence of the reflex but finds that

it is not always present. More recently McDowall (1934) has brought further evidence that, although the reflex is best shown when the venous and arterial pressure has been lowered, it may be present when the venous pressure is normal, and that the pressor fibres probably arise in the right auricle since painting the auricle with cocaine has the same effect as vagus section. He further suggests that when the venous pressure is high the vaso-constrictor centre is stimulated just as the cardiac sympathetic is stimulated as shown by Bainbridge and that this auriculo-pressor reflex may be of value in exercise in throwing the depressor reflexes from the aorta and carotid out of action.

Even when the vagi are cut, however, the blood pressure shows some signs of recovery. It might be suggested that pressor impulses pass up from the carotid sinus since Tournade and Malmejac (1930) found that faradic stimulation of the sinus nerve causes a rise of pressure especially if the vagi are cut. They have also found that stimulating of the nerve of the carotid sinus may cause a rise of blood pressure. This is supported by Danielopolu, Marcu, Proca and Aslam (1932). Heymans (private communication), however has been unable to find any evidence of such pressor fibres nor is it possible to demonstrate any fall of blood pressure on cocainising the sinus in circumstances when the vago-pressor reflex is present (McDowall, unpublished). Since the recovery is absent if the sinus has been previously cocainised, it must be considered that the recovery is due to the loss of the normal and depressor impulses from the sinus and to release of the vaso-constrictor centre.

It is probable that the vagus acts as the pathway of pressor impulses set up in other regions than the auricle, for de Waele and Van de Velde (1933) have found that the rise of blood pressure brought about in the cat as a result of occlusion of the portal vein does not occur if the vagi are cut. This reflex they suggest arises from the hepatic artery. It is present in the cat, lamb, and cock, but not in the rabbit and dog, guinea pig or pig. Bochefontaine and Bouceret (1877) also obtained a rise of blood pressure from the parietal pericardium.

The rise of blood pressure which results from the application of local irritants to vessels and from occlusion of a peripheral artery which of recent years has been studied particularly by Hess (1930) is dealt with at a later stage in this review.

RELEASE OF THE VASOCONSTRICTOR CENTRE. It was early recognised that section of the vagi would cause a rise in blood pressure, even after the administration of atropine to abolish the cardiac effects of the section. The view that impulses passed up the vagi to restrain the

vasomotor centre was put forward by Pavlov (1879) and the subject has subsequently been studied by a number of workers: Bayliss (1893), Reed, (1925), Thompson and MacDick (1928) and by Hammet (1932). In 1885, Sewall and Steiner reported a similar rise from section of the depressor nerve in the rabbit and no doubt the results recorded in relation to section of the vagi are really results from section of depressor fibres from the aorta and heart.

A very large amount of work, especially by Hering, Heymans and their pupils, has also been done in relation to the release of the vasoconstrictor centre as a result of loss of the depressor impulses from the carotid sinuses, which can readily be thrown out of action by occluding the common carotid arteries, by applying local anesthesia or denervation of the region. In each case there is a rise of blood pressure which is associated with constriction of all the vessels and organs which have been studied. Reference may be made to the list of tissues innervated from the sinus or to the monographs by Heymans (1933) and Koch (1932). It now seems reasonably certain that the difficulty to obtain a rise of blood pressure on section of the depressor nerve by Bayliss (1893) and by Pilcher and Sollmann (1914) were really due to the then unrecognized activity of the carotid sinuses which tend to prevent the blood pressure from rising when the aortic nerves or the vagi are cut. Provided conditions are otherwise suitable, it is a comparatively easy matter to demonstrate a rise of blood pressure from removal of the aortic and sinus impulses if one or the other have been previously thrown out of action. In all such experiments it is necessary to avoid respiratory complications by carrying out the experiments under artificial respiration. The release naturally fails to show itself if other circumstances are present which tend to stimulate the vasoconstrictor centre. If for example, the arterial and venous pressures are raised by placing the animal in the head down position or by the rapid injection of a gum saline into the blood stream, the rise of blood pressure may sometimes not be seen (McDowall, 1934). Such facts suggest that the normal depression of the vasoconstrictor centre by impulses from the aorta and sinus are thrown out of action by circumstances which raise the venous pressure, but the evidence is not yet complete. The release of the centre may, on the other hand, be accentuated by the local application of heat or any other form of sensory stimulation.

*The effect of surrounding temperature on vasomotor reactions.* It has been pointed out, especially by Rein (1931), that the temperature of the surroundings of an animal during an experiment may make a very

considerable difference to the vasomotor reactions obtained. Cold, for example, tends to cause vasoconstriction especially of the skin and of the splanchnic area. Many of these effects have hitherto been recognised as simple vasomotor reflexes such as those by Stewart (see "Vasoconstrictor Reflexes"), but the experiments of Rein, who has measured the flow of blood through different regions with a special stromuhr, and of Barcroft and Marshall (1923), who has made observations of the effects of cold on man, indicate that the effects produced are very general and from the work of Cramer (1916), of Cannon, Querido, Britton and Bright (1927) and of Crowden and Pearson (1928), the change may be considered to be associated with the secretion of adrenaline. Heat, on the other hand, has the opposite effect and, as well recognised, produces local reflexes. The more general effects may, however, be realised in a Spring (January, February) cat (Armitage, McDowall and Mathur, 1932) if gentle heat is applied after the aortic and sinus nerves have been thrown out of action (McDowall, unpublished). By such means, the blood pressure may be caused to fall as much as 75 per cent of its previous value. It may be assumed that the fall of blood pressure is prevented by nervous action and that in order to prevent the fall, the vasomotor centre is thrown into considerable activity. This may account for the well appreciated temporarily enervating effect in man of lying in the sun and emphasises the importance of carrying out experiments on the vasomotor system under known thermal conditions, both as regards external and internal environment. Much of this subject is, however, as yet little understood.

VASO-DILATOR MECHANISMS. The state of our knowledge on this subject is such that it is not possible to give any general classification of these mechanisms. It is, however, convenient to divide them into: (1) general dilator mechanisms, and (2) local dilator mechanisms. The general dilator mechanisms are those which stimulate the vasodilator centre and inhibit the vasoconstrictor centre and cause a fall of blood pressure. The local dilator mechanisms are those by which blood vessels of localised areas become opened up, the blood pressure being unaffected or even raised from increased activity of the vasoconstrictor centre.

*General dilator mechanisms.* These may be taken, like vaso-constrictor mechanisms, to consist of a vaso-dilator centre (using the term in a purely conventional sense and without presuming that it may be something more than the apex of a reflex arc), with afferent and efferent pathways.

*The vasodilator centres.* The first experiments which suggested the existence of a vaso-dilator centre are those of Laffont (1880) who found that destruction of the floor of the fourth ventricle interfered with the dilatation of the liver which he obtained when stimulating the first and second thoracic nerves. In the process of stabbing he also obtained a dilatation. It had also been observed that the depressor nerve is active after removal of all the brain above the medulla but not if the medulla is removed (Cyon and Ludwig, 1866). The existence of a centre was suggested by Ostroumov (1876) and by Tschirwinsky (1896) from the study of vaso-motor reflexes.

The centre was more accurately localised by Ranson and Billingley (1916) when exploring the floor of the fourth ventricle of the cat by the unipolar method. In the extreme posterior part of the fourth ventricle, just lateral to the obex, they found a point which, if stimulated, always gave a fall of blood pressure. This point is only 3 mm. from the vaso-constrictor centre.

The subject was re-investigated by Scott and Roberts (1923–24) who confirmed the essential points. Scott (1924) subsequently demonstrated that the fall of blood pressure usually produced by slow stimulation of the central end of the sciatic was unobtainable if the depressor point was cauterised or was painted with strychnine.

It is suggested that there is a vaso-dilator centre in the substantia reticularis grisea in the upper part of the medulla, since marked degeneration of the region has been found post-mortem in a patient in whom the blood pressure rose from 165 to 209 mm. Hg (Nordman and Müller, 1932). How far the rise might have been due to other causes is difficult to decide.

It must also be assumed that the centres concerned especially with the control of the skin vessels are in close connection with the heat regulating centre. Section through the pons is stated to cause a rise of blood pressure presumably the result of cutting off a higher dilatation centre (Porter and Storey, 1907).

Petroff (1931) has put forward evidence that the dilator centre like the constrictor centre is stimulated by carbon dioxide.

*Spinal vaso-dilator centres.* Studies in animals and in man in which the spinal cord has been severed suggest the existence of spinal centres or at least of local reflex arcs through the cord.

It was originally noted by Goltz (1874) that erection of the penis could be produced reflexly in a spinal dog if the organ was stimulated. Similarly he found that stimulation of the sciatic nerve could bring

about dilatation of the skin of the opposite paw. This was subsequently confirmed by Massuis and Vanlair (1875), Ustimowitsch (1887), and Thayer and Pal (1888).

Many of these observations have been confirmed on patients with injury to the spinal cord.

Ott (1879) showed also that reflex dilatation to heat could still be obtained after section of the cord above the 9th dorsal. He suggests therefore that the dilator centres for the hind limbs lie between this level and the 1st lumbar below which section of the cord has no effect.

*The effect of higher centres.* The phenomena of blushing and of erection indicate that the higher centres influence the vaso-dilator local mechanism, and many attempts have been made to demonstrate the effect of stimulation of the cerebrum.

A fall of blood pressure has been described by Bochefontaine (1876), by Stricker (1886), and by Bechterew and Mislawsky (1885). In some instances a remarkable fall of pressure was obtained on stimulating the sigmoid gyrus in the cerebrum adjoining the second primary convolution.

By far the most dramatic effect of the higher centres in causing dilatation is the phenomenon of fainting which is still a problem which is little understood. It is characterised by a profound fall of the arterial blood pressure which may be preceded by and is accompanied by intense pallor. Giddiness or complete loss of consciousness may occur. Typically the fainting occurs then the subject sees a disagreeable sight. The vessels affected are those of the muscles and it may be those of the splanchnic regions. The pallor of the skin indicates that these vessels are not concerned except in a compensation constriction. That the skin pallor is not merely due to draining of blood from the skin is seen from the fact that even when the fall of blood pressure has been recovered from the pallor persists. This I have observed in students who have witnessed animal experiments for the first time. In this connection, however, it is interesting to note that Dastre and Morat (1884) and also Langley (1925) observed constriction of the vessels of the ear of the rabbit occasionally when the aortic depressor nerve was stimulated.

The classical observation of John Hunter when bleeding a patient from the median basilic vein that the venous blood turned arterial in colour immediately prior to a faint indicates that limb vessels other than those of the skin are concerned.

The subject has been made a subject of study by Lewis (1932) who points out that the condition affects both the heart and the vessels.

The heart is slowed and although atropine causes a recovery in rate, the blood pressure remains below normal and consciousness is incomplete.

Many psychological explanations of fainting have been given. Probably the best is that it represents biologically a psychological escape from distressing surroundings. Lewis (1932) uses the term vaso-vagal but is doubtful if this so completely describes the state as sympathetic inhibitory for we know that any method of reducing sympathetic activity causes an apparent increase in vagus activity. Other forms of sympathetic paralysis or excessive parasympathetic action under conditions of extreme emotion are also common.

*The efferent pathway.* The pathway of vaso-dilator fibres down the cord appears to be by way of the antero-lateral columns (Ranson and Billingsley, 1916).

They emerge from the central nervous system in several ways, by special nerves, by the posterior nerve roots and by the sympathetic.

Typical special vaso-dilator nerves are the chorda tympani and the nervus erigens.

We have already noted, page 98, that Schiff from his experiments on the cervical sympathetic suggested the possibility of the occurrence of vaso-dilator nerves but that the chief proof came from C. Bernard who observed that when the chorda tympani was stimulated the venous blood became bright red. In 1912, however, Barcroft and Müller found that even when the secretive activity of the gland was paralysed by atropine the stimulation of the chorda still caused an increased oxygen usage. This has been used as an argument that the metabolic products are really responsible for the dilatation but Bayliss points out that the degree of dilatation is in excess of the oxygen used, a fact which indicates that a nervous dilatation exists apart from any dilatation produced chemically, while Anrep and Evans (1920) found that the dilatation of the tongue vessels which also occurs on stimulation of the chorda fibres in the lingual nerve (Vulpian, 1875) is not accompanied by any increased oxygen usage. It does not appear to have been pointed out in this connection that these fibres in the chorda tympani are essentially sensory. On the other hand, Dale (1906) found that when the secretion produced by sympathetic stimulation was paralysed by ergotoxine, the vaso-dilatation was also abolished. The amount of secretion produced by sympathetic stimulation is, however, very much less than that produced by stimulation of the chorda and the observation of Bernard (1858) in relation to the colour of the blood suggests also that the dilatation is in excess of the oxygen usage.

It is not to be presumed, however, that the products of metabolism do not also cause additional dilatation; indeed, the early experiments of Frey in his attempt to prove the occurrence of vaso-dilatation showed the point excellently. He studied (1876) the venous outflow during simultaneous stimulation of the chorda tympani and the sympathetic which causes vaso-constriction and found that by such stimulation their effects could be made to balance each other. After all stimulation had ceased, however, there occurred a dilatation which suggested that the sympathetic and vaso-dilators acted on different parts of the vascular system. This is more easily explained by the suggestion that the later vaso-dilatation is maintained by the metabolic products which have been produced in the gland during the stimulation. Bernard, Wittich and Heidenhain (quoted by Dastre and Morat, 1884) found that vessel dilatation preceded secretion.

It was shown by Bernard (1859) that the effects of chorda stimulation could be balanced by stimulation of the cervical sympathetic and later Anrep and Cybulski (1884) demonstrated that a similar balancing out of effects could be brought about by simultaneous stimulation of the hypoglossal and lingual nerves to the vessels of the tongue.

Gruber (1915) has pointed out that the excitability of the fibres concerned with secretion and dilatation are different.

The occurrence on stimulation of the nervus erigens of a true dilatation of the arteries of the penis first described by Eckhard (1863) has never been called in question, but whether or not these are also the sensory fibres and the reaction is what would now be called an antidromic one, does not appear to have been investigated. He and also Loeb (1870) have demonstrated that the tympanic branch of the glossopharyngeal has a function in relation to the parotid gland similar to that of the chorda on the submaxillary.

The idea that all parts of the body are supplied by vaso-dilator nerves was not generally accepted for many years, most workers in this field being engaged in attempting to disentangle the very mixed results which were obtained on stimulation of mixed nerves such as the sciatic. This nerve carries also vaso-constrictors to the hind limb, and unless special precautions are taken, stimulation of these fibres masks the effect of the vaso-dilators.

The first demonstration of vaso-dilators in the sciatic appears to have been made by Dogiel (1871) who obtained an increased venous flow and a rise of temperature in the hind limbs on stimulation of the sciatic after curari. To Goltz (1874) however, who used both electrical and

mechanical stimulation we owe the establishment of the idea of two kinds of fibres in the nerve. A similar view based on the effects of different strengths of stimuli had been put forward the year before by Onimus and Legros (1872) in regard to the effects of stimulation of the cervical sympathetic but this has apparently been overlooked or was considered of no value as a result of the observation of Chauveau on the horse, who obtained quite negative results. The idea was at first not generally accepted and Putzeys and Tarchanoff (1874) who had worked in Goltz' laboratory concluded that the dilatation could only be obtained after fatigue of the constrictor mechanism. Massuis and Vanlair (1875) succeeded in demonstrating dilatation with a weak current and Ostroumoff (1876) in Heidenhain's laboratory showed definitely that the dilators are more excitable than the constrictors. Weak tetanising or induction shocks at intervals of five seconds were found to cause dilatation while stronger or more rapid currents produced vaso-constriction. Bradford (1889) obtained vaso-dilatation of the kidney on stimulating the splanchnic nerve with a slow stimulus.

Such facts are in accordance with the observation of Gaskell (1878) who found that mechanical stimulation of a muscular nerve was particularly effective in producing vaso-dilatation.

Sewall and Sanford (1890) carried out similar experiments in man in which they recorded the finger volume. They found that strong electrical stimulation produced vaso-constriction but that weak stimulation caused a dilatation. They did not find that the nature of the stimulus mattered. On stimulation of the ulnar nerve dilatation was usual. These workers also point out, however, that they have made the general observation that on electrical stimulation of nerves constricted vessels tend to dilate and dilated vessels to constrict. This observation may prove to be of considerable importance in relation to the humoral transmission of nervous impulses.

Goltz (1874) also carried out experiments on sciatic nerves which had been cut some days before. He observed that whereas stimulation of the sciatic nerve when freshly cut caused a diminution in the redness of the skin of the toes and a lowering of the temperature as measured by a thermometer placed between the toes, the same stimulation of the nerve if cut two days before had the opposite effects. These results were confirmed by the work of Ostroumoff (1876) using changes in limb temperature as his criterion, by Kendall and Luchsinger (1876), and later by Bernstein (1877), Edgren (1880) and others. The constrictors usually lose their excitability three to four days after section of the

nerve (Dziedzul, 1880). Some dilator nerves have been found to remain excitable until the ninth (nervus erigens) and eleventh (chorda tympani) days after section, although the corresponding constrictor nerves die much more rapidly.

About the same time a number of investigators studied the effect of temperature on results obtained by stimulation of the peripheral end of the sciatic. Lepine (1876) and Bernstein (1877) demonstrated that vaso-dilatation could be well seen if the vessels were previously constricted by cold. Lepine (1876) and also Edgren (1880) emphasise, however, that the effect of temperature is primarily on the nerve itself.

All these results were fully confirmed by Bowditch and Warren (1886) using the plethysmograph which had been invented by Mosso (1874). They point out, however, that the effect of cold is not so marked as the work of previous workers had led them to suspect and they found that the latent period of stimulation was longer for the dilators than for the constrictors and that the dilator effect lasted longer and was less prompt in onset than the constrictor. These effects never neutralise but succeed each other, but the dilators are more readily exhausted. The results correspond well with those of von Frey (1876) in stimulating the dilators and constrictors to the salivary glands.

This difference of latent period has not been found for vaso-dilators and vaso-constrictors in the dog's tongue, but has been confirmed for the penis (Anrep and Cybulski, 1884; Piotrowski, 1887).

Later on the subject was further investigated by Ellis (1885) who found that if the total stimulation is constant the tendency to contraction increases as the number of induction shocks per second. Howell, Budgett and Leonard (1894) in investigating further the effect of temperature on nerves concluded that the vaso-constrictor nerves are more susceptible to changes in temperature than the vaso-dilators. The former were completely blocked at 3°C.

It is, however, possible, by using different stimuli, to demonstrate the characteristics of both kinds of nerves at various temperatures (Asher, 1909).

Head and Bayliss (1923) also found that after section the vaso-dilators of the radial nerve of the cat recovered before the constrictors. Stepanow (1922) found it possible to demonstrate the vaso-dilators in the frog by cocaine, KCl, drying, and mechanical compression. The effects are reversible. Stepanow points out that the vaso-dilators are supposed to be of later origin than the constrictors.

The above facts appear to indicate clearly the existence of two kinds

of fibres. The facts, however, do not prove beyond doubt that these different fibres actually exist as it is possible that the same fibres may produce different effects. Chauchard (1925) in Lapique's laboratory and Oinuma (1925), who have measured the chronaxie of apparent constrictors and dilators have not been able to demonstrate any difference in excitability. Further, Stepanow (1922 b) has found that if the current of action be recorded it can be shown that fibres from the sympathetic and from the spinal ganglia reproduce equally well frequencies of stimulation up to 100 per second. He concludes, therefore, that the different vasomotor effects depend on the qualities of the peripheral apparatus and not upon the nerves. As pointed out by Martin and his co-workers in relation to the effects of afferent stimulation the procedures which cause dilatation really reduce the number of active fibres.

The different effect of stimulation may then depend simply on the predomination of the constrictor fibres. In view of the large volume of new work on the humoral transmission of nerve impulses these points are of great interest. It might even be suggested that whether a vaso-dilator or vaso-constrictor substance is produced at the nerve endings depends on the number of impulses reaching them.

Many facts which have been stated in relation to the mammal also hold for the frog, in which mechanical stimulation of the sciatic causes dilatation of the vessels of the web of the hind foot (Ellis, 1885). Stronger stimulation causes a constriction unless the nerve is cut and allowed to degenerate (Ellis, 1885; Oinuma, 1911; Pearce, 1913; Krogh, 1921; Koenigs, 1912). Doi (1920) and also Stepanow (1922) have later confirmed these points. In the frog the fibres which pass to the sciatic apparently arise from the 8th and 9th dorsal.

The final proving that the sciatic did contain dilator nerves leads to the natural question regarding their origin from the central nervous system.

Stricker (1876) showed that mechanical or electrical stimulation of posterior nerve roots of the 6th and 7th lumbar nerves brought about vaso-dilatation of the hind limbs, but a number of well-known workers (Cossy, 1876; Vulpian, 1878, and Kuchsweller, 1885) failed to confirm the observation which has, however, been amply confirmed since, by Morat (1892), Bayliss (1900), Ranson and Wightmann (1922), Langley (1923), Dennig (1924), Hinsey and Gasser (1930).

Hasterlik and Biedl (1893), Bonuzzi (1885), Laffont (1882), Bornezzi (1887) and Werziloff (1896) found that stimulation of the 4th to the 7th

lumbar and 1st sacral nerves caused a rise of temperature in the hind limb of the dog, while the venous pressure increased.

In 1901 the problem was extensively studied by Bayliss. He was unable to distinguish between the efferent vaso-dilator fibres and the ordinary in-going afferent fibres. They degenerate when the posterior root ganglion is removed but do not degenerate if the roots are cut between the cord and the ganglion. Bayliss therefore gave to these dilator impulses the term *antidromic* because they pass in a direction opposite to that of the ordinary sensory impulses. Confirmation of such antidromic dilator fibres has been given by Hinsey and Gasser (1930) who showed that the fibres concerned in the posterior root were the same as those concerned in Sherrington's contracture, i.e., the contraction of a voluntary muscle sensitised by denervation, by Bonuzzi (1885) in relation to the supply of the fore-limbs, and by Langley (1923). It was later shown by Mosonyi (1927) that atropine did not prevent the action of the antidromic impulses even after ether, chloroform, and curare.

In the frog Doi (1920) found that stimulation of the posterior roots caused a dilatation of vessels in the web. This was confirmed by Krogh, Harrop and Rehberg (1922) and by Karlik (1929) who found that the dilatation so produced did not interfere with the effect of constrictor sympathetic stimulation. Krogh, Harrop and Rehberg (1922) consider that only a limited number of large capillaries respond. Bena (1930) could obtain confirmation in the guinea pig while Oinuma (1911) was unable to demonstrate dilator fibres in the 8th and 9th roots although he got a positive result with the sciatic. Hasama (1931), however, describes remarkable dilatation on stimulation of the 7th, 8th, and 9th posterior roots in the toad. This he found was abolished by the application of nicotine to the posterior root ganglion.

Ranson and Wightman (1922) consider that the dilator nerves connect with the sensory cells in the spinal ganglion like sympathetic nerves and may either enter from the posterior roots or pass through the anterior roots and reach the ganglia by a detour through their respective thoracic and sacral nerves. It might then be considered that the fibres are not really antidromic except in part of the root. Mislawsky and Bistrenin (1905) on the other hand maintained that the dilator fibres pass out in the posterior roots and have no connection with the cells of the spinal ganglion and do not degenerate centrally when the roots are cut. It is not possible, however, to obtain any inhibition of the crossed extension reflex by a stimulation of antidromic fibres as

might be expected if the antidromic fibres were true sensory nerves (Forbes, Smith, Lambert, Caveness, and Derbyshire, 1933). On the other hand, it was shown clearly by Feldberg (1926) that the dilators of the ear running in the auricularis ventralis and dorsalis correspond in distribution to the sensory distribution of these nerves.

The fibres which pass out by the posterior roots apparently join the ordinary mixed nerves, possibly by first passing to the sympathetic chain (see below), and are distributed with them as is shown by the effect of slow galvanic stimulation. Bayliss (1901 and 1902) found that if the posterior roots are cut and time allowed for degeneration, stimulation of such a mixed nerve as the sciatic no longer gives dilatation even when a suitable stimulus is used. In view of the evidence of dilator fibres in the sympathetic this conclusion of Bayliss is doubtful.

Bayliss quotes an interesting experiment by himself and Head (1923). At a stage of regeneration of the radial nerve of the cat at which only the so-called protopathic sensory fibres were present, stimulation of the peripheral end caused an obvious dilatation of the paw. The constrictor fibres do not recover till some weeks later. This nerve contains no motor fibres for voluntary muscle, and it sometimes happens, apparently, there are no vaso-constrictor fibres in the normal nerve. The absence of constrictor fibres can be assured by removal of the stellate ganglion and, after time is allowed for degeneration, a pure vascular dilatation was obtained on stimulation of the sensory nerves to the limbs. In the case of the hind limbs, 5th, 6th, 7th lumbar and 1st and 2nd sacral are concerned (Bayliss, 1900). The vessels concerned are, apparently, both those of the skin (Doi, 1920) and also those of the muscles (Metallinos, 1925).

It is interesting in the light of later research to remark that several old experiments had demonstrated that sensory nerves had a dilator effect. We have already referred to the lingual which contains sensory fibres to the tongue. The trigeminal also is a vaso-dilator to the face and interior of the nose, the eye (Vulpian, 1875; Schulten, 1884; Morat and Doyon, 1892; Tschalussow, 1913). The general significance of these observations was not at the time appreciated.

Dilator nerves to the veins have also been found to pass out by the posterior roots (Horiuchi, 1924).

The facial and the glossopharyngeal also carry dilator fibres (Vulpian, 1885) to the posterior part of the tongue, uvula, and tonsils.

The superior laryngeal has been found to carry dilators, e.g., to the larynx (Cyon, 1897; Hedon, 1906; Mislawsky, 1906; Sinakewiez, 1906;

François Franck and Hallion, 1908; Ossokin, 1914). The later observers also found dilator fibres to the thyroid in the inferior laryngeal nerve.

Of recent years, Ken Kure (1931) and his collaborators have emphasised the existence of the efferent fibres which pass out by the posterior roots and have suggested that they are parasympathetic fibres which have a motor action on the gut. A criticism of this conclusion has appeared in a recent review by McSwiney (1931) in this journal.

The sacral nerves supply the rectum and descending colon (rabbit and cat) Langley and Anderson (1895) and also the bladder (Mislawsky, 1906).

The sexual organs have a mixed source of supply. According to Weekers (1906) the prostate is supplied from the inferior mesenteric ganglion through the hypogastric plexus. The penis receives its vaso-dilator supply from special dilator nerves, as discovered by Eckhard (1863), the nervi erigentes, which arise from anterior roots of the 1st, 2nd, and occasionally the 3rd sacral nerves and reach the organ by the hypogastric plexus. Stimulation of the nerves causes erection which has been shown to be a purely vascular phenomenon (Eckhard, 1863 et. seq.). François Franck (1895), however, suggested a further supply from the lumbar ganglion through the hypogastric plexus to the pudendal but the lumbar origin could not be confirmed by Langley and Anderson (1895–96) although Laffont and Vitzore (1879) obtained dilatation of the glans penis through stimulation of the external pudendal. The mammary glands in the dog appear to be supplied by the lumbar nerves (Laffont, 1879; Laffont and Vitzore, 1879).

In connection with the efferent pathway it is interesting to recall that Gaskell appears to have been of opinion that the dilator fibres to muscles reached the mixed nerves from the anterior roots.

*Vaso-dilator fibres in the sympathetic.* There is a considerable volume of evidence that vaso-dilator fibres may be contained within the sympathetic chain, at least in certain parts of the body, and this is all the more remarkable since there is evidence that the same parts obtain vaso-dilator fibres from other sources. This is true of the head and neck. Dastre and Morat (1880) (Bernard's successor) found that in the dog vaso-dilator fibres pass out by the 2nd to 5th thoracic and up by the ansa and cervical sympathetic to supply the head and neck. They remark that had Bernard chanced to use a dog instead of a rabbit for his original experiments, he would have been more impressed by the vaso-dilator action of the sympathetic than by the vaso-constrictor function. A similar conclusion may be arrived at from the early

experiments of Schiff (1859), already quoted. Dziedziul (1880) using the same procedures which we have already described for the sciatic confirmed the observation by finding that the vaso-dilators did not degenerate so rapidly as the vaso-constrictors. The presence of two categories of fibres readily accounts for the results of Vulpian and of Prussak (1868) who occasionally obtained dilatation of the ear. In the rabbit the dilatation is certainly absent (Feldberg and Schiff, 1926). Isergin (1894) obtained a similar dilatation of the tongue on stimulating the cervical sympathetic with weak currents. In 1907 Carlson found that in eight out of twelve cases, stimulation of the cervical sympathetic caused increased flow from the vein of the submaxillary gland in the cat which in such circumstances produces a copious secretion. This was subsequently confirmed by Barcroft (1908), Gruber (1915), Asher (1910), Gesell (1919) and is supported by the finding of McLean (1908) that adrenaline causes an increased flow from the submaxillary vein.

Carlson (1907) found also that the results were affected by the previous injection of atropine which has a nicotine-like action (Langley, 1878) on the superior cervical ganglion. Bayliss (1908) as we have stated found that the dilatation was small compared with that produced by stimulation of the chorda tympani, but as he points out this may be due to the fact that vaso-constrictors are stimulated at the same time.

Heidenhain (1872 b) and also Langley (1893) obtain flushing of the mouth on stimulation of the cervical sympathetic. Gaskell (1916) attributed this result to the fact that the region is freely supplied with glands. The metabolites might be responsible for the dilatation. It is difficult to disprove this contention in some regions but in others, such as the ear, the occurrence of sufficient increased metabolism seems unlikely.

On stimulating the thoracic sympathetic Dastre and Morat (1880) obtained a dilatation of the hind limbs but on stimulating the sympathetic below the diaphragm a constriction was produced.

Dale (1906) however showed that after the administration of ergotoxine to throw the constrictors out of action, stimulation of the abdominal sympathetic caused a dilatation of the hind limbs and stimulation of the splanchnic dilatation of the intestine.

It will be remembered that Bayliss (1901–02) obtained no dilatation from stimulation of the sciatic after section and degeneration of the posterior roots from which the nerves take origin. If the abdominal sympathetic contains fibres to the lower limbs stimulation of the sciatic should still cause dilatation unless the fibres go by other paths, possibly

by the arterial walls. Bayliss (1901–02) was unable to obtain dilatation of the hind limbs from stimulation of the abdominal sympathetic even before time had elapsed for degeneration of the dilator fibres, while Gaskell (1878) also obtained dilatation of the muscles from stimulation of the anterior roots of the sciatic but not from the abdominal sympathetic. Bayliss, however, worked on the cat which appears to be different in this respect from the dog. We have already noted this difference in relation to the cervical sympathetic.

Langley (1923) on the other hand found that stimulation of the lumbar sympathetic causes flushing of the pad of the cat's foot after, as a rule, a preliminary pallor. This flushing was found to be reduced by atropine.

Burn (1932) also supports the earlier work of Dale (1906) and has shown in the dog but not in the cat not only that stimulation of the abdominal sympathetic dilates the vessels of the hind limbs but that the fibres are cholinergic, i.e., they probably produce acetyl-choline for the dilatation is increased by eserine and abolished by atropine (Dale, 1934). Thus, we must consider that these fibres are very different from those thrown into action by the depressor nerve which are not prevented from acting by atropine. The experiments of Dunlop and Waterston (1934) suggest that the sympathetic fibres may be concerned in the fall of blood pressure produced by stimulation of somatic afferent nerves, since they find that such depression is cholinergically produced.

It should be noted that the action on the hind limbs of acetyl choline injected intravenously is prevented by atropine (Hasama, 1931). Kibjakow (1931) working on the posterior roots has found that their stimulation causes a dilator substance to be liberated into the blood or Ringer's solution perfused through them. If this is correct why atropine fails to abolish the action of the aortic depressor nerve is difficult to explain. Dale and Gaddum (1930) have suggested that the acetylcholine is produced in a more intimate relation to the vascular muscle than the atropine can succeed in reaching. A consideration of this aspect of the humoral transmission of nerve impulses is outwith the scope of this review. A summary by Dale (1934 b) is available in his Linacre Lecture.

According to de Waele, van der Velde, and Braeye (1933) the dilatation of the paw which occurs on stimulating the peripheral end of the posterior roots between the cord and the ganglion really depends on afferent fibres and is dependent on the presence of the superior abdominal ganglion. Similarly Bacq, Brouha, and Heymans (1933) found that removal of the lumbo sacral sympathetic brought about complete

loss of the vascular reflexes in the hind limb from the carotid sinus. Mere removal of the thoracico-lumbar outflow did not interfere with the reflexes, although the blood pressure had completely recovered from the operative procedure (Bacq, Brouha, and Heymans, 1932).

In the frog, also, dilator fibres appear to reach the hind limbs also by way of the sympathetic (Speranskaja-Stepanowa, 1925).

We may then conclude that the dilator fibres which pass out by the posterior roots are somehow connected to the lower part of the ganglionic chain although how this attachment is made is as yet uncertain.

The idea that the abdominal sympathetic contained dilators to the hind limbs is not really new and is described in the older work of Dastre and Morat (1884), Ostroumoff (1876), Puelma and Luchsinger (1878). According to Dziedziul (1880) and to Maximowitch (1886) the fibres to the hind limbs join the lumbo-sacral plexus direct and pass from this to the sciatic and anterior crural without coming into contact with the sympathetic chain.

All these facts bring into consideration the observations of Dale and Richards (1927) that small doses of adrenaline cause vaso-dilatation; but whether the fact is related to any nervous action is at present unknown.

*Vasodilators to viscera.* The idea that the sympathetic supplied the viscera with vaso-dilator fibres was first put forward by Laffont (1880) but no dilatation of the intestine could be found by Lehndorff (1908). Bradford (1889) also obtained a dilatation of the kidney from splanchnic stimulation. Bradford and also Johansson (1890) obtained a fall of aortic pressure from slow rates of stimuli applied to the splanchnic nerves and some mixed nerve roots (9th thoracic to 1st lumbar, especially 11th, 12th, and 13th dorsal). Bunch (1899) also found dilator fibres in the splanchnic and obtained secondary dilatation from stimulation of the lower thoracic and upper lumbar roots, especially with weak currents. Similar results were obtained by François Frank and Hallion (1896 a) with the 7th thoracic to the 2nd lumbar. On stimulation of the 6th thoracic to 1st lumbar, François Frank and Hallion (1897) obtained a dilatation of the pancreatic vessels, while Schafer and Moore (1896) record one instance of a dilatation of the spleen on stimulation of the splanchnic. Biedl (1897) found that vaso-dilatation of the suprarenal occurred when the splanchnic was stimulated.

A dilatation of the mesenteric vessels in the frog was found on sympathetic stimulation by Trazaska and Chrzonezewsky (1899).

Thompson (1928) obtained a fall of pressure on stimulation of the

right splanchnic nerve, but it is necessary that the constrictor action of the nerve should first be exhausted by prolonged stimulation as described in the case of the sciatic by Putzeys and Tarchanoff (1874). This is confirmed by Liddell and Sherrington (1929).

It seems therefore to be proved that in the dog at least the abdominal sympathetic contains some vaso-dilator fibres like the cervical sympathetic. It is interesting to observe also that Dastre and Morat (1880) state definitely that the vaso-dilator fibres to the head arise from anterior roots while Laffont (1882) found that dilator fibres to the hind limbs arise from the 2nd to 4th lumbar anterior roots and it is generally agreed that the nervus erigens arises from the anterior roots of the 2nd and 3rd sacral (rabbit) and 1st and 2nd sacral (dog), and it will be recalled that Gaskell (1916) obtained a dilatation of the vessels of muscles on stimulation of anterior roots, while Bradford (1889) also obtained dilatation of the kidney from anterior roots. It seems probable that those stimulated by Gaskell (1916) were from the sympathetic (see "Vasodilators to Muscles," below). Here, however, a new problem shows itself, for some sympathetic fibres are adrenergic, i.e., produce an adrenaline-like substance at their nerve endings and we know that adrenaline in small doses dilates muscle vessels. We may, therefore, ask: Are there such fibres additional to those which are cholinergic? Research on these points is proceeding in many laboratories and a definite answer will perhaps be given soon. Since this review was written a paper has appeared by Rosenblueth and Cannon (1934) who conclude that the vasodilator system is probably constituted as follows (1) sympathetic dilators primarily to striated muscles, (2) parasympathetic dilators distributed to special localized regions, (3) dorsal root dilators mainly to the skin and viscera. As will be seen below the suggestion that the sympathetic supplies the muscles has much evidence to support it especially the fact that adrenaline dilates the vessels of muscles.

It is, however, interesting to remark that the fibres which are thrown into action by stimulation of the central end of the aortic and presumably the carotid depressor which have a special path by the posterior roots and which are not thrown out of action by atropine are the reciprocals of the vaso-constrictors of the anterior roots (Bayliss, 1908) while there is evidence of another system of vaso-dilators from the sympathetic which is not. The experiments of Bacq, Brouha and Heymans (1932) suggest however that even those which pass out by the posterior root are connected with the sympathetic but there is much in this field still to be cleared up. In view of the apparent difference in different

animals, experiments on man are important. Lewis and Pickering (1931) studied the vaso-dilatation produced in a cool arm or leg by exposing the body to a warm temperature. By comparing the reaction in Raynaud's disease with the effects of anesthesia of the ulnar nerve, they concluded that human sympathetic nerves contain vaso-dilator as well as vaso-constrictor fibres.

*Vaso-dilators in the vagus nerves.* There is considerable evidence that the vagi carry vaso-dilator fibres, especially to the gut. This was described by Latchenberger and Deahna (1876) in regard to the stomach, by François Franck and Hallion (1896) in relation to the liver and intestine after the administration of atropine, and by Bunch (1899) who, however, generally obtained negative results in the cat and rabbit. The dilatation of the pancreas is by François Franck and Hallion (1897), of the liver by Cavazzani and Manca (1895), of the kidney by Japelli and Tria (1907). The effect on the kidney is, however, contradicted by Pearce (1914) and the effect on the liver by Griffiths and Emery (1930) but Carnot, Gayet and Merklin 1930 obtained a fall of portal pressure when the vagus was stimulated. Anrep (1916) obtained dilatation of the pancreas on stimulating the vagus but did not consider this to be vascular. Bayliss (1923) also could not find any evidence of dilator fibres in the vagus of the cat and rabbit. On the other hand Barry (1933) obtained a fall of blood pressure from stimulation of the peripheral end of the vagus after excluding cardiac effects.

All stimulation experiments have the disadvantage that they do not exclude the effect of metabolic changes also produced locally. Indeed, the same difficulties apply which have already been referred to in relation to the action of the chorda tympani on the submaxillary gland.

*The regions innervated in the depressor reflexes.* When the action of the aortic depressor nerve was first discovered by Cyon and Ludwig (1866) they put forward the idea that the dilatation was wholly splanchnic but by clamping the aorta high up Heidenhain and Grützner (1878) soon showed that the dilatation was by no means confined to this region. This was confirmed by Sollman and Pilcher (1912) and supported by Porter and Beyer (1900) who cut the nerve supply to the splanchnic region and by Stelling (1867) who cut the spinal cord at the level of the third thoracic segment and still obtained a fall of blood pressure. Smirnow (1886) also showed that this did not abolish the reflex.

Anrep and Starling (1925) and Heymans and Bouckaert (1929) demonstrated that the arteries of the head were also concerned in the reaction to a general fall of blood pressure.

That the capillaries take part in the reaction has been suggested by McDowall (1922) on the grounds that the absolute or percentage fall of blood pressure due to histamine was reduced by stimulation of the aortic nerve. This has subsequently been confirmed microscopically on the mesentery of the rabbit by Kock and Nordmann (1928) although the authors are inclined to look upon the dilatation as passive rather than active. In the frog the capillaries take part in the dilatation which occurs in stimulating the posterior roots (Doi, 1920).

Bayliss (1908), it will be recalled, considered that the efferent pathway of the depressor reflex was antidromic while in the pads and toes of the cat also it was found by Langley (1923) that the capillaries rather than arteries were concerned in antidromic dilatation.

It is stated also that the coronary arteries are dilated (Hochrein and Gros, 1932).

The fact that the splanchnic region is greatly concerned has been amply confirmed by Dreschfield (1867), Bayliss (1893), François Franck and Hallion (1896), Dastre and Morat (1884), Bunch (1899), Jarish and Ludwig (1926 a), Kock and Nordmann (1928). Bayliss (1893) who carried out a most extensive plethysmographic study of the question found dilatation in all regions which were examined and that in the limbs both the skin and the muscle were affected. This has been confirmed by Fofanow and Tschalussow (1913). The dilatation of the muscles was also noted by Gaskell (1878) and confirmed by Jarisch (1926) in the gastrocnemius of the rabbit. François Franck (1899) had already noted that an eviscerated animal showed a fall of blood pressure; that the pulmonary vessels are also affected was also shown by Sharpey Schafer (1920) assisted by the author. The vessels of the submaxillary are also affected (Bayliss, 1893; Asher, 1909), and also of the tongue and mucous membrane of the nose (Bayliss, 1908; Fofanow and Tschalussow, 1913; Tschalussow, 1913; Martin and Mendenhall, 1915), but it has been found that the dilatation is best seen if the antagonistic cervical sympathetic has been previously cut. Incidentally this experiment shows all the more clearly that the dilatation is the result of dilator stimulation rather than of reduction of constrictor tone. A dilatation of the kidney is described by Roever (1869) who observed redness and swelling, by Bradford (1889) using the plethysmograph and by Sollmann and Pilcher (1912) using the innervated perfusion method. The latter applied the same method to the spleen with a similar result.

A dilatation of the ear of the rabbit has been described by Bayliss (1893), Simon (1929), and Weber (1908), but Dastre and Morat (1884)

and also Langley (1925) have described instances in which the ear vessels constricted when the general blood pressure fell as a result of depressor stimulation. The vessels of the penis are also said to dilate in stimulation of the aortic nerve (Tournade and Malmejac, 1933).

The only contrary results which have been obtained have been on the ear of the rabbit (Dastre and Morat, 1884; Cyon, 1899; Bayliss, 1893; Weber, 1908; Simon, 1929; Langley, 1925). But Bayliss (1893) points out that a short period of dilatation can be found if adequate means are taken; for example, an examination by thermopile, but this view is not supported by Langley (1925). The cause of these diverse results is probably due to the fact that the fall of blood pressure produced by the depressor activates the carotid sinuses in those animals in which the carotid sinus mechanism is very active.

Much work has been done on the effect of stimulating the carotid sinus by raising the pressure in it or by stimulating its nerve and by cutting off the impulses from the sinus by reduction of its internal pressure, especially by Heymans (1933) and his collaborators.

The areas shown to be affected are best shown in tabular form:

*Intestine:* Moissejeff (1926), Tournade (1930), Heymans, Bouckaert and Dautrebande (1931).

*Extremities:* Sollman and Brown (1912), Tournade (1930), Rein (1931), Heymans, Bouckaert and Dautrebande (1931–2).

*Head:* Heymans and Bouckaert (1929).

*Skin:* Holtmeier (1927), Gaisbock (1928), Koch and Simon (1928).

*Nose:* Binet and Gayet (1929).

*Thyroid:* Rein (1932).

*Liver:* Heymans, Bouckaert and Dautrebande (1930).

*Cerebral vessels:* Negative results, Heymans and Bouckaert (1932).

*Coronary arteries:* Hochrein and Keller (1932). These observers found a constriction of these vessels during a fall of pressure in the sinus. In view of our knowledge of the coronary control this result requires confirmation.

*Retina:* Gollwitzer-Meier and Schulte (1932).

*Lungs:* Tournade (1932). This author considered the results to be secondary to changes in the general circulation, but in view of the experiments of Sharpey-Schafer (1920) in relation to the depressor nerve this seems doubtful.

*Veins (femoral):* Heymans and Bouckaert (1930), Heymans, Bouckaert and Dautrebande (1931); (*mesenteric*): Fleisch (1931); (*intestinal*) Gollwitzer-Meier and Schulte (1931).

*Muscles:* Rein (1930).

*Penis:* Tournade and Malmejac (1933).

There is still a very large amount of work to be done on the effects produced by vaso-dilatation brought about by the stimulation of somatic

nerves. In this respect the work of Rein (1930) which showed that different methods of stimulating the centres may produce different blood distributions is most suggestive.

*The vaso-dilator fibres to muscles.* The general problem of the control of the muscle vessels has been generally neglected, but in view of their enormous capacity there can be little doubt that they must be capable of producing enormous changes in the circulation if they become dilated. It is stated that a third of the total blood in the body is in the muscles (Spael, 1883; Ranke, 1871).

The idea that the innervation of the vessels of the muscles is different from that of the skin or intestine arises from the work of Heidenhain and Grützner (1878) who found that a stimulation of a centripetal nerve, for example the radial or saphenus, brought about a vaso-dilatation in the muscles of the thigh. Much further work both in relation to nerve stimulation and to the effect of adrenaline which actively dilates the vessels of muscles has shown that this view is probably correct. The most important direct observations on this point were made by Gaskell (1877–78) who studied the blood-flow from muscles.

The only stimulation of roots which would bring about dilatation of the muscular vessels of the hind limbs was that of the anterior roots (i.e., well below the sympathetic outflow). He also found that stimulation of the motor nerves to a muscle caused vaso-dilatation but in discussing his results was inclined to favour the possibility that the metabolites formed are chiefly concerned in this. He does not, however, definitely exclude the possibility that vaso-dilator nerves are concerned for he observed under the microscope vaso-dilatation in the mylohyoid muscle of the frog on stimulation of its nerve, even after the injection of small doses of curari which prevented any muscular contraction. Mechanical stimulation was found to be particularly effective. Larger doses of curari abolish the vascular effects also. If the muscle is tetanised an increased venous outflow occurs quite apart from the initial increase which occurs from the driving out of blood by the contraction. This has also been pointed out by Burton-Opitz (1902) and by Tschvewsky (1903).

It has also been pointed out by Sadler (1869) that if shortening is prevented the increase of blood flow still occurs.

There is still, however, much work to be done on this important problem for Heidenhain (1877) and Gaskell (1878) also found that there was marked vascular dilatation in the extensor muscles of the thigh when the abdominal sympathetic is cut, and this has been confirmed by

Spallitto and Consiglio (1897) plethysmographically by Bayliss and by Hartman, Blatz and Kilborn (1919), taking the increased temperature in the muscles as a criterion. The only possible fallacy is that the dilators were stimulated by the section but the effect was too prolonged. In exercise the evidence is complete but outside the limits of this review that metabolites and nerves act together to produce the enormous increase in blood-flow which is known to occur in active muscle. In 1892 Kaufmann showed that the blood-flow through the masseter muscle of a horse may be increased five times by allowing it to masticate normally.

*Afferent stimuli to the vaso-dilator centres.* It may in the present state of our knowledge be said as a general statement that the stimuli which affect the vaso-dilator centres in the medulla are those which stimulate the vagus centre. By far the most important impulses are those which pass up from the aorta[1] and the carotid sinuses, but the present evidence suggests that in certain circumstances at least impulses pass up by way of any afferent nerve. In studying the effect of stimuli the cardio-inhibitory effect must be excluded by the administration of atropine or by section of the vagi. The evidence is now very complete that at each beat of the heart impulses pass up from these regions and bring about an inhibition of the vaso-constrictor centre and stimulation of the vaso-dilator centre, bringing about generalised vaso-dilatation and a fall of blood pressure. The evidence regarding the nature of the stimuli from the aorta and carotid sinuses and their pathways is exhaustively dealt with in recent monographs by Heymans (1933), by Hering (1932) and by Koch (1931).

The suggestion of Anrep and Starling (1925) that a rise of pressure in the circle of Willis might bring about generalised dilatation has not been supported by the subsequent experiments of Florey, Marvin and Drury (1928). There is little doubt that many of the results obtained by Anrep and Starling were due to changes in the carotid sinus, the importance of which was not then appreciated.

In all such experiments it is necessary to exclude, by the use of small doses of curari or preferably by opening the chest widely (merely having it under artificial respiration is insufficient unless a closed system is used) the effect of increased respiration as first pointed out by Vincent and

[1] Throughout this review the term aortic depressor nerve is used to designate what has been commonly called the depressor nerve. The term aortic nerve was that first used to describe it by Wooldridge and avoids confusion with the other depressor nerves.

Cameron in 1916, i.e. before the importance of washing out carbon dioxide from the centres was appreciated. There can be little doubt also that many of the discordant results which have been obtained have been due to lack of knowledge regarding the aortic and particularly the carotid nerves. If these are put out of action the animal becomes much more sensitive to depressor impulses, effects being produced by stimuli, mechanical, electrical, and thermal, which are otherwise ineffective (McDowall, 1933).

It is sometimes possible by stimulation of the central end of the vagus in a cat to produce a shock-like state especially if the aorta and sinus are denervated and the animal is cold. Chloral is stated to cause predisposition to a fall (Ludwig and Cyon, 1866; Heidenhain and Grützner, 1878) ether and chloroform (Hunt,1895), and narcosis generally (Martin, Franklin and Hield, 1920) pyridine (Brunton and Tunnicliffe, 1894), and the barbiturates, avertin, and paraldehyde (Lacey, 1932). All these, it is noted, reduce sensory impulses. The reverse appears to occur when the nervous system is unduly irritable as a result of removal of the parathyroid and thyroid glands. Vincent and Thompson (1928 d) consider that the parathyroid limits the secretion of a pressor substance since the phenomenon is not affected by the injection of calcium salts. It is however doubtful if the calcium was given long enough time to become effective in their experiments which were done on ordinary decerebrate animals. It should perhaps be added that however unsatisfactory anesthetics are in the investigation of vasomotor reflexes the use of decerebrate animals is open to many objections especially to the loss of carbon dioxide and the cutting off of the higher sympathetic centres. A general study of the action of anesthetics on the vasomotor reflexes was made by Richet and Dublineau (1930). Most workers now use chloralosane or chloralose. Under the latter the sympathetic is particularly active (Vincent and Thompson, 1928 a) but in both the degree of anesthesia is important.

Some regions of the body are particularly liable to cause fall of blood pressure, especially mechanical stimulation of the anus and vagina. According to Belfield (1882) the effect disappears in the dog when the splanchnic nerves are cut. Similar effects have been described by surgeons in man and also to section of the spermatic cord.

Mechanical stimulation of the peritoneum or abdominal viscera has a similar effect also in an innervated perfused limb (Tournade and Malmejac, 1933 b). In the frog Goltz (1864) found that tapping the intestines caused a generalised dilatation.

Busquet (1914) found that pinching the foot might cause a fall of femoral pressure as a result of a reflex, while jarring the operating table had a similar effect. Winkler (1902) found that thermal stimuli also acted reflexly.

The circumstances in which electrical stimulation of the central end of a mixed nerve may bring about a fall of blood pressure were studied in detail by Knoll (1885) and especially by Reid Hunt (1895) who investigated reflexes by the methods which had previously been used for the demonstration of the presence of vaso-dilators in mixed nerves (see "The Efferent Pathway"). He found that stimulation after cooling of the nerves to about 4°C. or stimulation at a certain stage in regeneration (also Vincent and Cameron, 1916), weak stimulation or mechanical stimulation also by Knoll (1885) and Vincent and Thompson (1928 b) especially of the muscles causes a fall of blood pressure. This has also been pointed out by Brunton and Tunnicliffe (1894), Kleen (1889), Vincent and Thompson (1928 b), and later by McDowall (1933), using the blood pressure and the vascular dilatation in an innervated perfused limb to exclude any possible effects of chemical substances produced by the stimulation. Hunt (1895) was unable however to bring about any fall by chemical stimuli or by varying the rate of stimulation. This was, however, found by Gruber (1917) who stimulated at the rate of 4 per second and obtained a fall of blood pressure with a strength of stimulus which gives a rise if administered at 20 per second. Why Hunt did not get a fall with such stimulation is difficult to explain. It may be that his stimulus was too strong or anesthesia too light. Vincent and Ogata (1918) and also Scott (1923), Vincent and Thompson (1928 b) and Dunlop and Waterston have however confirmed the importance of slow stimulation.

Hunt (1895) and also, previously, Asp (1867) obtained a fall of blood pressure especially from muscular nerves, while Tengwall (1895) studying the subject and paying great care to the dissection obtained a fall on all occasions. The fall was sometimes succeeded by a rise. There is no doubt, however, that a fall may be obtained from a cutaneous nerve such as the radial (McDowall, 1933). Vincent and Thompson (1928 b) who studied the subject in great detail were unsuccessful in attempting to associate depression especially with afferent nerve fibres, but point out that some branches of the sciatic may give a fall of blood pressure when stimulated by a stimulus which if applied to the whole sciatic nerve would cause a rise. Bradford (1889), however, described that they could only obtain a fall of blood pressure from the intercostal nerves.

Sometimes it was preceded by a rise (Bradford and Dean, 1894). Auer and Meltzer (1913) and Burton Opitz (1916 and 1917) have also obtained a fall of blood pressure from stimulation of the central end of the thoracic sympathetic and splanchnic in the dog but not in the cat. Foster (1893) emphasised the importance of the state of the centre in such experiments. This no doubt is profoundly affected by the anesthesia and the carbon dioxide of the blood.

According to Latchenberger and Deahna (1876) and also to Hunt (1895, 1918) after repeated stimulation a rise of blood pressure may be replaced by a fall, just as in similar circumstances, which suggest fatigue in the arcs, repeated stimulation may cause cardiac slowing instead of acceleration (Roy and Adami, 1892; McDowall, 1932).

In all experiments on the subject it should, however, be pointed out that, in order to obtain the best results, it is necessary to denervate the aorta and the carotid sinus. When these buffer nerves are removed stimuli previously ineffective may cause a profound fall of blood pressure (McDowall, 1933).

When the constrictors are depressed by narcotics the dilators are more liable to prevail. In support of this view is the fact described by Bacq, Brouha, and Heymans (1932) that a spontaneous or induced agitation may cause a marked fall of blood pressure or even syncope in a cat whose thoracic lumbar sympathetic has been removed and whose blood pressure has recovered.

It would appear, then, that the demonstration of reflex dilator action from mixed nerves depends on the fact that the excitability of pressor fibres differs from that of depressor fibres. Actually measurements of the chronaxie of vaso-dilator and vaso-constrictor fibres do not show that they have any difference in excitability, as might be expected. It must be considered that the slower stimulation simply stimulates fewer fibres (Chauchard, 1925; Oinuma, 1925). Another explanation, however, is possible and has been put forward by Martin (1914, 1915) and his co-workers, indeed, it seems from further work more probable, namely, that a vaso-dilatation is produced when a small number of afferent impulses reach the central nervous system and a vaso-constriction when a larger number is sent in. This subject has been discussed already in a review by Ranson (1921). The view that both vaso-dilators and constrictors are stimulated by ordinary faradic stimulation of the central end of the sciatic has since been strongly supported by work of Dunlop and Waterston (1934) who have shown that the fall of blood pressure which succeeds the rise is accentuated by eserine.

The existence of two afferent vaso-motor pathways in the spinal cord has definitely been demonstrated by Ranson and Hess (1915) and by Ranson and Billingsley (1917). Earlier work by Dittmar (1870) had shown by section that the afferent pathway for the pressor reflexes did not lie in the posterior columns, while Sherrington described the pathway in the anterior columns. Bikeles and Zbyszewski (1918) located the efferent dilator pathway in the same region. Ranson and Billingsley (1917) described the afferent dilator pathway as lying in the antero-lateral regions, while the afferent constrictor pathway is at the apex of the posterior horns.

*Reflex dilatation of muscle vessels and related reactions.* The evidence is very complete that stimulation of practically any sensory nerve causes a dilatation of muscle vessels.

We have already referred to the work of Heidenhain and Grützner (1878) who found that stimulation of the radial nerve causes a dilatation of the vessels of the thigh. Similar experiments were subsequently described by Wertheimer (1893–94) who stimulated the sciatic and the brachial plexus and later by Edwards (1914) who found an increase of blood flow in the femoral vein when the splanchnic nerve was stimulated. François Franck (1893, 1899) stimulated the anterior crural.

The fact that on stimulation of an afferent nerve an increased flow from the muscle vessels occurs at the same time as constriction of the splanchnic vessels also suggests in the same direction.

Isergin (1894) found that on stimulation of the sciatic a dilatation of the lingual vessels might occur. How far these results are due to direct nerve section or to the adrenaline which is probably liberated by such stimulation does not appear to have been investigated. This may explain also the statement by Wertheimer (1893–94) that when the superficial vessels are contracted by cold the muscle vessels are dilated, for we know that cold calls forth a secretion of adrenaline. All such work requires repetition with the adrenal glands removed. This may be said especially of the results of Heidenhain (1870) and of Wertheimer (1894) who found the application of cold to the skin brought about a dilatation of muscle vessels.

To provide blood for the muscles the vessels of the skin and intestine are constricted and possibly those of the lungs (Azzi, 1920). That there is a physiological antagonism between the limb vessels and those of the splanchnic region was originally pointed out by Bayliss and Bradford (1894). A similar antagonism between the portal vessels and the skin was pointed out by Dastre and Morat (1882) who studied

venous outflow, and observed that when one was constricted the other was dilated, while Anrep (1912) emphasised also the importance of the splanchnic region in the regulation of the blood supply of the body generally. Bayliss (1923) however could not confirm Dastre and Morat, but Langley (1924) frequently found flushing of the feet to be associated with pallor of the stomach and intestines, while Pavlov (1878) considered that cutaneous vaso-constriction indicated intestinal vaso-dilatation.

Rein (1930) (1931) using his thermostromuhr found that the muscle vessels dilate when the vasomotor centre is stimulated reflexly as by lowered carotid sinus pressure, but not if the centre is stimulated directly by carbon dioxide. The result is difficult to interpret for both procedures presumably cause a secretion of adrenaline, which dilates muscle vessels. Probably the increased carbon dioxide in the blood affected his result. Jarisch (1925) in studying the gastrocnemius found that on depressor stimulation the muscle dilatation was produced by sympathetic inhibition and not by antidromic stimulation. At the same time the vessels of the ear may constrict (Langley, 1925).

It must then be considered possible that in the muscle as in other regions there may be two distinct dilator mechanisms, one of which is the reciprocal of the vaso-constrictor mechanism and one which is concerned with the blood supply during exercise.

*The reciprocity of the centres in reflex vaso-dilatation.* The evidence is now fairly complete that the vaso-dilatation which results from stimulation of the aortic or carotid depressor nerves is the result not only of inhibition of the vaso-constrictor mechanism as originally imagined by Cyon and Ludwig (1866) but also a stimulation of a vaso-dilator mechanism. The latter view was first put forward by Ostroumoff (1876).

Bayliss (1893) has shown quite clearly in the case of the action of the depressor nerve that both processes are concerned. He cut the posterior roots to the hind leg of the dog and still obtained a dilatation, thus showing that there was an inhibition of the normal constrictor tone. The stimulation of the vaso-dilator mechanism he demonstrated in the submaxillary gland after section of the cervical sympathetic which supplies the vaso-constrictors. These results are supported also by Asher (1917). In such circumstances depressor stimulation still causes an increased flow of blood from the gland. Similar results have been obtained by Fofanow and Tschalussov (1913) on the hind limb on cutting the vaso-constrictor fibres. The early experiments of Goltz and Freusberg (1874) and Goltz and Gergens (1875) suggest that vaso-dilator stimulation is more effective than loss of constrictor tone.

A similar reciprocity between the vaso-constrictor and vaso-dilator mechanisms has been shown to occur when the carotid sinus is stimulated or has its normal stimulus withdrawn. Bacq, Brouha and Heymans (1932) have also shown that after all the sympathetic has been removed in cats a fall of blood pressure in the carotid sinus still causes a rise of blood pressure. This I have confirmed but in order to do so I have found it necessary to inject gum Ringer solution in order to maintain the blood pressure which falls as a result of the removal. They have also shown that the vaso-dilators as much as the vaso-constrictors take part in the reaction.

Where the reflexes concerned are proprioceptive and for the maintenance of blood pressure at a constant level during rest, there can be little doubt that reciprocity between the centres exists. It should not, however, be assumed—as has been done, apparently, by all writers on this subject—that this reciprocity applies also to the effect of stimulation of any mixed nerve, or, what is more important, in exercise. Indeed, there is good evidence that it does not apply. Bayliss (1908) on the one hand sought to prove the general application of the principle of reciprocity, which he established for the depressor reflex, but he could not prove it for pressor reflexes. At least he has published no records of it unless he established an artificial dilator tone by previously stimulating the central end of the depressor nerve. He finally admits that the inhibition of dilator tone does not play much part in pressor reflexes, such as are obtained from the median or sciatic nerves. Since at that time the carotid sinus had not been discovered, it was not possible to study the pressor effects of a fall of blood pressure in an uncomplicated way. Martin (1914, 1915, 1922), Ranson (1917) and their associates, supported by Gruber (1917, 1918), have on good evidence sought to disprove the general principle of reciprocity of the vascular centres from a study of the effects of stimulation of mixed nerves. Their conclusions do not contradict those of Bayliss (1908) to the extent that they appear to think although they prove that there is no reciprocity in regard to the pressor reflexes from mixed nerves. It should perhaps be said in fairness to Martin and to Ranson that, although Bayliss found little evidence of the existence of reciprocity in pressor reflexes, he believed in its existence, a conviction somewhat natural from the very definite results obtained from the depressor reflex and the then recent discovery of reciprocal innervation by Sherrington.

In 1914 Martin and Lacey studied the threshold of stimulation necessary for the production of pressor and depressor effects from

mixed nerves, and they were struck by the fact that the threshold for the pressor reflexes was thirty-three times that of the depressor which is that for ordinary spinal reflexes. It was not affected by curari (Martin and Stiles, 1914) but pyridine and chloral cause a rise to be converted into a fall (Brunton and Tunnicliffe, 1894). Later, Martin and Mendenhall (1915) using the reaction of the nasal mucous membrane after the method of Tschalussow as an indicator, noted the existence of marked grading and latency in the pressor compared with the depressor reflexes. They therefore suggested that in the pressor reflexes there was a depressor component, which had to be overpowered before the rise of blood pressure occurred and explained the effect of cold and degeneration in eliciting depressor impulses as being due simply to a cutting down of the volume of impulses passing to the vasomotor centres. Martin and Jacoby (1922) pointed out that even a large amount of weak stimulation, such as the immersion of the lower half of the body in a bath at 42°C. in nine out of eleven experiments was followed by rise of blood pressure. Supporting the same idea is the interesting observation made in another connection by Vincent and Thompson (1928) that a given stimulus if applied to the whole sciatic may cause a rise but if applied to one of its twigs may cause a fall. They have emphasised also that the secretion of a chemical substance is responsible for the rise but like many others have overlooked the fact that many of the mechanical stimulative procedures used cause a production of histamine and the secretion of adrenaline.

Heidenhain and Grützner (1878) also pointed out the importance of amount rather than degree of stimulation in producing pressor results and pointed out that even tickling or blowing on extended areas might cause very large rises of blood pressure, although strong (even destructive) stimuli were without effect. If, however, as pointed out by Bayliss (1893) the depressor nerve is stimulated, its effects may be counterbalanced by stimulation of a pressor nerve up to a certain point when a strong depressor action cannot be overpowered (Martin and Stiles, 1916). Samson Wright also in stimulating the central end of the vagus pointed out that although the pressor effect might balance the depressor, after the stimulation the depressor effect might become apparent. Similarly Martin, Franklin and Hield (1920) found that mild stimuli—tactile, auditory, or thermal—provided they did not arouse any mental alertness, produced an increase in the ear volume of the rabbit. In the same kind of way, warm water applied to the skin causes vaso-dilatation, but as soon as it becomes hot and painful, there is constriction (Munk,

1910; Hirchfeld, 1912; Stewart, 1911). On the other hand, the cornea which has only pain receptors only gives pressor responses (Wilson).

The view that vaso-dilators are stimulated at the same time as the vaso-constrictors is suggested by the rebound or further rise of blood pressure which may occur after cessation of stimulation of an afferent nerve in a decerebrate animal (Marcu, 1929). This suggests that during the stimulation the simultaneous stimulation of depressors kept the blood pressure from rising. When the blood pressure falls after sensory stimulation it may be considered that the dilator action has been the more prolonged. It is interesting in this respect, too, that Vincent and Thompson (1929) pointed out that monotonous music has a similar effect on man.

Ranson (1921) accepts the explanation of the fact that the effect depends on the volume of impulses passing up the nerve and not, as Hunt (1895) and others imagined, on the stimulation of different fibres in the nerve, each group connected to the dilator or constrictor centres, and this view is supported by the action of pyridine and narcotics which do not reverse but paralyse the sensory side of reflex arcs, but is contradicted by Tengwall (1895) who obtained only a fall from the stimulation of afferent muscular nerves. Langley (1912) compared the reflex effect of stimulating the vagus and internal saphenus in the rabbit. He found that their pressor action favoured by curari but their depressor action favoured by strychnine; but the depressor action was never reversed. He records also that in a decerebrate animal tactile stimuli cause a rise of pressure but urethane converts it into a fall, a fact easily explicable on Martin's view that the afferent impulses have become reduced.

We may then conclude that the usual faradic stimulation of a mixed nerve excites both the vaso-dilators and the vaso-constrictors and that there is no reciprocity of the centres in this variety of stimulation.

Ranson and Billingsley (1916) found that the efferent pressor and depressor pathways in the cord are separate and considered that the stronger stimulus was able to take the path of greater resistance, i.e., the pressor path, for reasons which, however, Martin (1922) has subjected to considerable criticism on the general principles of conduction in nerve. Both Ranson and Martin are, however, in complete agreement regarding the absence of such reciprocity. It is interesting to remark that McDowall (1931) has recently pointed out the absence of reciprocity of the cardiac centres in stimulation of a mixed nerve, and has produced evidence that, when the heart rate is increased—say, by stimulation of the sciatic nerve—the vagus centre is also stimulated although its effect

is masked until the acceleration is passed off when marked slowing similar to normal vagus restraint occurs and may persist for some time. In certain circumstances slowing only will occur. On the other hand it is relatively easy to demonstrate the occurrence of reciprocity of the cardiac centres in relation to the aortic and carotid reflexes. For example, cardiac slowing may be produced by stimulating the central end of the aortic depressor nerve although both vagi are cut, presumably from inhibition of the sympathetic (v. Brucke, 1917). This I have often confirmed.

The position in the cardiac and vascular responses is identical in regard to reciprocity of the centre, and it may be concluded that although reciprocity is absent from reflexes produced from the stimulation of mixed nerves, it is present in relation to the purely vascular reflexes by which the blood pressure levels are maintained. The functional value in relation to the latter reflexes is obvious.

The simultaneous stimulation of the dilator and constrictor centre when a mixed nerve is excited has no doubt also functional importance in relation to the maintenance of dilator tone (see "The Value of General Vaso-dilatation" above), just as the stimulation of the vagus as well as the sympathetic has, as pointed out by McDowall (1934) a considerable value in relation to the cardiac output.

It may be concluded that, while the experiments of Martin (1914 et seq.) and his co-workers show definitely that there is no reciprocity of the centre when a mixed nerve is stimulated, it is not so well proved that this effect is not brought about by different fibres joining the separate paths shown just as definitely by Ranson and Billingsley (1916) to exist in the cord.

*The value of general vaso-dilatation.* There seems every reason to believe that there is a fall not only of the arterial pressure but also of the venous pressure. This occurs presumably as a result of the widespread dilatation of vessels, arterioles, capillaries and veins. The fall of venous pressure is comparatively easy to demonstrate in the cat when the aortic nerve is stimulated but commonly a rise of venous pressure is obtained in the rabbit or hare.

In regard to responses to a fall of pressure in the carotid sinus no change in venous pressure is recorded in the central end of the renal vein (Heymans, Bouckaert and Dautrebande, 1930) or in the external jugular, as commonly recorded, but if the venous pressure is high in some cases a rise is obtained (McDowall, 1934, unpublished). A rise is obtained commonly in the portal vein (Heymans, Bouckaert, and Dautrebande,

1930–31) presumably the result of a constriction of veins. It does not appear that the effect of a rise of pressure in the carotid sinus has yet been studied.

The very irregular results obtained suggest that much more work is required in this field as the results may very well be much affected by the peripheral resistance and blood volume in relation to the capacity of the peripheral vascular bed.

It may be considered, as pointed out by Bayliss (1923), that in order that the heart should be relieved when confronted by a high arterial pressure it is desirable that the depressor mechanism should affect the cardiac filling, otherwise the effect of diminishing peripheral resistance would be frustrated. This view presumes, as is commonly assumed, that the general depressor mechanisms exist for the protection of the heart. It is very doubtful if this assumption is justified, since we know that in physical exercise the blood pressure rises in spite of the dilator mechanisms. Nor is the heart any the worse provided it is healthy. We must, then, consider the possibility that the vaso-dilator mechanisms may serve other purposes than the relief of the heart.

One of these is heat regulation, but this obviously cannot apply to the deep vessels. It would be reasonable to suggest that the vaso-dilator mechanism is primarily concerned with blood volume, and the greater the vaso-dilator tone the more blood is available in the vessels at a low pressure for use by the muscles in exercise when the vessels of unused areas are shut down. This brings vaso-dilator tone in relation to vagus tone, which we know depends on similar if not identical afferent fibres from the aorta and carotid sinus, and which is similarly thrown out of action in exercise. The probable value of vagus restraint to the animal is probably not only protective but in relation to cardiac filling (McDowall, 1926).

*Local vaso-dilator reflexes.* These reflexes are different from the general vaso-dilator reflexes in that the dilator effect is limited to the regions where the stimulation is made. They are found both in the skin and the internal organs and may be associated with general constriction elsewhere which results in a rise of blood pressure.

Reflexes of this type were first described by Bernard (1858) who observed dilatation of the submaxillary vessels when the tongue was stimulated, and by Snellen (1858) who observed that stimulation of the posterior auricular nerve not infrequently gave a dilatation of the ear of the rabbit. In 1866 Lovén repeated and studied more carefully the experiment on the ear, with similar results. He also stimulated the

dorsalis pedis nerve and obtained dilatation of the femoral artery while stimulation of the sensory nerves of the face caused dilatation of the external maxillary artery.

Bradford (1889) supported these observations by finding dilatation of the kidney on stimulation of the central ends of the dorsal roots (11th to 13th) of the kidney area. Heating of the skin has been found to have a similar effect (Burton-Opitz and Lucas, 1909). The alleged beneficial effect of cupping in kidney disease must be considered to be due to a reflex of this nature as, indeed, is some of the beneficial effect of poultices and fomentations. The same year François Franck described dilatation of the whole head when the mucous membrane of the nose was stimulated, while Dastre and Morat (1884) found that all parts of the head which receive vaso-dilator nerves by the cervical sympathetic show vaso-dilatation when the superior laryngeal nerve is stimulated. The reddening of the face of man if the upper respiratory passages are stimulated is well known. Massuis and Vanlair (1875) observed that stimulation of the central end of the sciatic caused a dilatation of the vessels of the paw of the opposite hind leg, but this result, however, obviously depends on the kind of stimulation used.

Similar experiments were described on the frog by Huizinga (1875) and by Gaskell (1877) who studied the mylohyoid muscle and by Edgren (1880) on the rabbit (François Franck and Hallion, 1896 and Busquet, 1917).

It is also well known that the placing of one hand in warm water will cause the other to dilate sometimes after a preliminary constriction (Stewart, 1911; Hess and Bermann, 1913; Müller, 1905; Sewall and Sanford, 1890).

The opposite effect, as we have seen in relation to vaso-constriction, occurs with cold even when there is sufficient anesthesia to prevent the patient from feeling the temperature (Tholozan and Brown Sequard, 1858; Stewart and Laffer, 1913; Stewart and Walker, 1913).

These responses to temperature are, however, more probably general than local. Local dilator reflex may be accompanied by local constrictor effects. Langley (1924), for example, found that stimulation of the central end of a hind limb nerve sometimes caused flushing followed by pallor in the other hind foot and slight pallor in the large intestine. The flushing of the foot is enhanced by strychnine.

Similar results may occur in man on electrical stimulation of the lower arm, but constriction may also be obtained (Maragliano and Lusona, 1889).

Bernard (1858) had in his early work remarked on the dilatation of the submaxillary vessels when the tongue is stimulated, a fact no doubt related to the stimulatory effect of the presence of food in the mouth. How far the fact described by Burton-Opitz (1917) that stimulation of the central end of the splanchnic nerve may cause a fall of blood pressure may represent a local dilator reflex of the Snellen-Lovén type is difficult to decide.

It is, however, a little difficult to explain the dilatation of the ear vessels described by Weber (1908) on stimulation of the sciatic and by Owsjanikow and Tschirjew (1872) and also by Lovén (1866) on stimulation of the brachial, the more so in view of the fact that other investigators have observed constriction (see "Constrictor reflexes"). It may be that due attention was not paid to respiration which is commonly stimulated by sensory stimulation and the loss of carbon dioxide peripherally may result in a constriction of the ear vessels or it may have been that the vessels may have been paralysed by rough handling or by the anesthetic and have been possibly dilated. The occurrence of passive dilatation has been shown to be possible by François Franck (1896) and by Bayliss and Bradford (1894). These latter investigators concluded that the rise of blood pressure brought about by stimulation of the anterior crural or the central end of the splanchnic caused passive dilatation because it disappeared if the splanchnic was cut and constriction of the splanchnic area prevented. Schäfer and Moore (1896) also refer to passive dilatation of the spleen when the blood pressure rises. I am inclined to believe that the possibility of passive dilatation has been exaggerated in the limbs for it is fairly easy to demonstrate that even when there is an enormous rise of blood pressure caused by occlusion of the carotid arteries and application of the vertebral clamp (McDowall, 1930b) there is very little dilatation of a skinned limb although a small dose of adrenaline will cause an enormous dilatation. In the experiments just referred to, section of the splanchnics would prevent the secretion of adrenaline.

There is good reason to include erection of the penis and possibly of the nipples as purely local dilator reflexes. The former certainly can be produced in animals and man in which the spinal cord has been injured.

Reactions very similar to Lovén reflexes arise from the blood vessels themselves.

It was first demonstrated by Heger (1887) on the rabbit and dog that peripheral injection of nicotine or silver nitrate into the femoral artery after ligature of the crural vein causes a rise of blood pressure. This was

shown to be purely a nervous effect and apparently to depend on the capillaries for localisation of the irritants to the larger vessels produced, rather remarkably, no effect. That the internal surface of vessels is sensitive to chemical irritants, however, is now generally admitted (Spallitto and Consiglio, 1892), and it is now common knowledge that, while puncture of a vein may be painless, puncture of an artery may be exquisitely painful.

The whole subject of reflexes of this nature has received considerable attention since it has been suggested by Hess (1930) that the rise of blood pressure which results from blockage of an artery to any part represents an attempt on the part of the body to supply the part with blood and may therefore be termed a "Nutrition" reflex. The subject was first studied by Latchenberger and Deahna (1876) who found that a fall in blood pressure in an artery resulted in pressor impulses passing up to the vasomotor centre and a local dilatation of vessels. It was, however, soon shown by Zuntz (1878) that it was the anemia of the tissues rather than the fall of pressure which caused the stimulation. Ludwig (1931) finds that if the circulation of three extremities is shut off, there is no rise of blood pressure in normal men, but there may be in vasolabile patients and those with essential hypertension. In the cat also a rise of blood pressure is not commonly produced by clamping the femoral arteries in circumstances when clipping the carotid with the sinus nerves intact causes a marked rise. Schilf (1924) however has found that stimulation of the peri-arterial tissue of the femoral artery causes increase of limb volume which is apparently reflex since it is abolished by section of the femoral nerve. General surgical opinion supports the view that most peri-arterial nerves are afferent.

De Waele and van de Velde (1933) are of opinion that the mere changing of the position of the limbs may give vasomotor reflexes.

The opening up of collateral paths when an artery to a part is occluded appears to depend at least to some extent on nervous influences, since after tying the artery the temperature of the part might rise temporarily above normal (Ferris and Harvey, 1925).

We may then conclude that while there is evidence that anemia of a part leads to a local dilatation of vessels, the effects of local products of asphyxia are sufficient to account for this, while the general rise of blood pressure is not sufficiently marked or easily obtained to justify its being designated by any special name. This conclusion is in accordance with the findings of Heymans (1929) and of Heymans, Euler and Dautrebande (1931–32) who point out that there is no rise of the general blood

pressure if nerve filaments are not compressed at the same time. I have also failed to obtain evidence of such a reflex. Cerebral anemia on which stress is laid by Hess must be considered in a very different category from anemia say of a limb.

*The axon reflex.* By this reflex is meant a dilatation of skin vessels which occurs in the neighbourhood of a point of local stimulation but which appears to depend on the axon of a sensory nerve. The term axon reflex was originally given by Langley and Anderson (1894) to a similar reflex response of the bladder to stimulation.

It was later used by Bruce (1910) in relation to the reddening of the skin produced by oil of mustard to the cat's conjunctiva. The reddening persisted for a few days after the Gasserian ganglion on the 5th nerve had been removed for neuralgia but after the sensory fibre had degenerated the reddening was no longer obtained. The main facts were confirmed soon afterwards by Janushka (1913) and by Bardy (1915). The latter states that nicotine abolishes the effect. Previous to Bruce's experiment it had been assumed that the reddening was due to some spinal reflex, for Speiss (1906) had observed that the reddening due to mustard oil could be prevented by the local application of cocaine, and that in regard to wounds, the more sensitive the better the healing.

The reaction probably affects capillaries as well as arterioles, for Krogh (1921 b) found that, while touching a capillary of the frog's tongue with a pointed glass rod caused not only local dilatation but a spread of the dilatation, the spread was absent if the nerves to the tongue had been previously cut and allowed to degenerate. The capillary reaction does not however appear to be concerned in the skin reaction of man. Goltz (1860) had at an earlier date noted the fact that skin dilatation took place after the nerves to a part had been cut an observation which has since been amply confirmed by Ebbecke and by Lewis and his co-workers who have shown the importance of the local production of dilator substances.

Considerable attention has of later years been paid to the phenomenon of the flare which is produced by stroking the skin of man with a blunt instrument. The phenomenon now known as the triple response was first described by Marey (1858).

If the skin, especially of the abdomen, is stroked, a white line appears. According to Serjent (1903) the occurrence of the white line is particularly liable to occur in adrenal insufficiency but the view has been definitely contradicted by Wright (1922) who has shown it to be present in many normal persons. Since each arteriole supplies a regular area

of skin, the production of a white line which is limited to the area of the stroke may be considered to be due to capillary constriction produced as a result of mechanical stimulation (Cotton, Slade and Lewis, 1917). This fact has been confirmed by microscopic observations of the superficial vessels and the fact that it is not affected by cutting off the blood supply to the part, e.g., the arm by an inflated armlet. Were the arterioles to constrict in such circumstances they would presumably drive blood into the capillaries and dilate them further. The reaction is obtained after all the nerves to the part have become degenerated.

If the stroke is stronger, the white line is replaced by a red line, which becomes bordered by two white lines which are more temporary. If the stimulus is stronger still, a general flare around the area of the stroke is produced (Müller, 1913; Cotton, Slade and Lewis, 1917; Ebbecke, 1917). A review of the general position in 1917 was written by Wheelan.

This flare, which in some persons is specially well marked, has been studied by Lewis (1927) and his collaborators in men and by Krogh (1929) and his collaborators in animals. Both workers have put forward their views in extensive monographs. It has been shown to be arteriolar and to depend on the local nerves. The nervous factor had been previously emphasised by Ebbecke (1917) who at the same time noted the importance of protein cleavage products. He obtained similar reactions by gentle stroking of internal organs such as liver and kidney, but not in muscle. The reddening tends to be patchy and irregular, as would be expected from the arteriolar distribution in the skin. The temperature of the skin rises, sometimes by 2°C. as a result of the increased blood flow. The area never becomes purple from loss of oxygen, showing that the blood flow is rapid. A white line may be produced on the flare by light stroking. If the upper arm is compressed until the distal part of the arm is cyanosed (Cotton, Slade and Lewis, 1917) or blue with cold (Carrier, 1922) heavy stroking will elicit a flare on a dark background, the result of more oxygenated blood being forced from the deeper vessels to the surface.

In persons with very susceptible skin, a wheal develops at the site of the stroke and is bordered by a typical flare which is produced reflexly. The inoculation of histamine into the skin produces a small blister surrounded by a flare such as might be produced by a gnat bite. A variety of chemical agents and ultra-violet light (Lewis and Zotterman, 1926) produce such a flare; indeed almost any strong chemical agent. It is suggested by Lewis and Grant (1924) Lewis, Grant and

Marvin, and by Lewis and Harmer (1927), that they all liberate the same chemical substance which, like histamine, produces a typical triple response. Heat produces a similar result with a central wheal or blister if sufficiently severe.

Reflex flare, such as that produced by cutaneous stimulation, has been shown to depend on nerves. Richardson and Wyatt (1918) and Krogh, Harrop and Rehberg (1922) abolished it by the application of cocaine and novocaine. It is abolished by section (Lewis, 1930; Lewis and Grant 1924). Krogh and Rehberg (1927) and his co-workers have studied the reflex in the rabbit ear and in the tongue and web of the frog. In the tongue the reaction is like that of mammals, but in the web it is more complicated. It is interesting at this point to remark that several early workers had noted the importance of nervous connections in local skin reactions. Rogers (1890) for example studied the response of the ear to warmth after sections of the nerve supply to the regions. It was found also by Samuel (1900) that the reactions and capability of the ear to withstand local infection were profoundly reduced by cutting its nerve supply.

The nervous path apparently passes from the subcutaneous tissue and not from the skin itself (Lewis, Grant and Marvin, 1927), a fact which may be related to the observations of Woollard (1926) that the medullated nerves to limb vessels run not only in the vessels but also in the adjacent tissues. A similar but less marked change dependent on nerves can be produced in the rabbit's ear by the application of histamine (Grant, 1930).

Krogh and Rehberg (1927) have put forward evidence that the venules are actively dilated in reflex flare. They measured the pressure in the venules of the skin of the knuckles by means of the transparent Recklinghausen capsule. They found the pressure to be from 6 to 11 c.m. of water.

This, however, is contradicted by Lewis (1928) who points out that the values got by these authors are too low and that normal skin colour, not blanching, should be taken as the index. If this index is used upon skin placed at the level of the heart values of 50 to 60 mm. Hg are obtained in histamine flare. He therefore concludes that the venules may be passively dilated.

The failure of Stevenson and Reid (1915) to find any difference in the effect of mustard on the normal and anesthetised eye is no doubt explained by the fact that the mustard causes also a local capillary dilatation not necessarily dependent on nerves.

It has been suggested that a spinal arc is concerned in the flare (Rajka and Furth, 1924). Ebbecke (1917) had a similar view, but the experiments just referred to make it clear that this is not so. The view of Bruce (1910) is supported also by that of Breslauer (1919) who has studied the effect of mustard oil on the human skin. He has found that the reaction is unaffected in patients in which the corresponding part of the spinal cord has been destroyed or the posterior nerve roots and in fresh cases of section of the corresponding spinal nerve while it fails to appear after the nerves have degenerated.

## REFERENCES


ADRIAN, BRONK AND PHILIPS. J. Physiol. **74**: 115, 1932.
ADRIAN AND BUYTENDIJK. J. Physiol. **71**: 121, 1931.
ALLEN. Am. J. Physiol. **87**: 319, 1928.
ANREP. J. Physiol. **45**: 307, 1912.
ANREP. J. Physiol. **50**: 412, 1916.
Physiol. Reviews **6**: 596, 1926.
ANREP AND CYBULSKI. Quoted from Hoffman and Schwabb's Jahresb. **13**: 49, 1884.
ANREP AND EVANS. J. Physiol. **54**: Proc. 10, 1920.
ANREP AND SEGALL. J. Physiol. **61**: 215, 1926.
ANREP AND STARLING. Proc. Roy. Soc. B. **97**: 463, 1925.
ARMITAGE, MCDOWALL AND MATHUR. Quart. J. Exp. Physiol. **21**: 365, 1932.
ASHER. Ergebn. d. Physiol. **1**, B: 346, 1902.
Ztschr. f. Biol. **52**: 311, 1909.
Pflüger's Arch. **136**: 411, 1910.
Ztschr. f. Biol. **68**: 160, 1917.
ASHER AND LUESCHER. Ztschr. f. Biol. **38**: 499, 1899.
ASHNER. Wien. Klin. Wchnschr. **21**: 1529, 1908.
ASP. Ber. d. Sachs Ges. d. Wiss. *m/p. k. l.* **136**: 183, 1867.
ATZLER. Deutsch. Med. Wchnschr. **49**: 873, 1923.
ATZLER AND LEHMANN. Pflüger's Arch. **193**: 463, 1922.
AUBERT AND ROEVER. Pflüger's Arch. **1**: 211, 1868.
AUER AND MELTZER. Centralbl. f. Physiol. **26**: 1316, 1912.
AVELING AND MCDOWALL. J. Physiol. **60**: 316, 1925.
AZZI. Lo Spermentale **74**: 25, 1920.
BACQ, BROUHA AND HEYMANS. C. R. Soc. Biol. **111**: 152, 1932.
C. R. Soc. Biol. **112**: 208, 1933.
BAINBRIDGE. J. Physiol. **50**: 65, 1915.
BANCROFT. Am. J. Physiol. **1**: 477, 1898.
BARCROFT. J. Physiol. **35**: Proc. 29, 1907.
Respiratory function of the blood. 2nd ed., C. U. P., 1925.
J. Physiol. **36**: Proc. 53, 1908.
J. Physiol. **68**: 357, 1929–30.
BARCROFT AND FLOREY. J. Physiol. **68**: 181, 1929.
BARCROFT AND KATO. Phil. Trans. B. Roy. Soc. **207**: 149, 1916.

Barcroft and Marshall. J. Physiol. **58**: 145, 1923.
Barcroft and Müller. J. Physiol. **44**: 259, 1912.
Barcroft and Stephens. J. Physiol. **64**: 1, 1927.
Bard. Am. J. Physiol. **84**: 490, 1928.
Bardy. Skand. Arch. Physiol. **32**: 196, 1915.
Barry. Brit. Med. J. **2**: 372, 1933.
v. Basch. Ludwig's Arbeit., 1895.
Bayliss. J. Physiol. **14**: 303, 1893.
J. Physiol. **26**: 173, Proc. 30, 1901.
J. Physiol. **28**: 276, 1902.
Ergebn. d. Physiol. **5**: 319, 1906.
Proc. Roy. Soc. B. **80**: 339, 1908.
J. Physiol. **37**: 264, 1908.
The vasomotor system. Longmans, London, 1923.
Bayliss and Bradford. J. Physiol. **16**: 10, 1894.
Bayliss and Head. Quoted by Bayliss. The vasomotor system. Longmans, 1923.
Bayliss and Starling. J. Physiol. **17**: 120, 1894.
Beale. Phil. Trans. Roy. Soc. 611, 1860.
Bechterew and Mislawsky. Neurol. Centralbl. 194, 1885.
Beck. Phil. Trans. Roy. Soc. 213, 1846.
Belfield. Arch. f. Anat. u. Physiol. 298, 1882.
Bena. Pflüger's Arch. **224**: 258, 1930.
Benhamon, Marchioni and Nouchy. C. R. Soc. Biol. **100**: 456, 1929.
Benjamin and Rochat. Pflüger's Arch. **164**: 111, 1916.
Benjamin. Arch. nerdl. d. Physiol. **1**: 704, 1918.
Bergin. Arch. f. Anat. u. Physiol. **1**: 652, 1876.
Berkowitch. Jahresb. z. Anat. u. Physiol. **2**: 53, 1875.
Bernard. C. R. Soc. Biol. **3**: 163, 1851.
J. de Physiol. **1**: 233, 1858.
Leçons sur les liquides de l'organisme. Paris, 275, 310, 1859.
J. de Physiol. **5**: 383, 1862.
Bernard, Wittich and Heidenhain. Quoted by Dastre and Morat, 1884.
Bernstein. Pflüger's Arch. **15**: 289, 575, 1877.
Berthier, Deschamps and Halfern. C. R. Soc. Biol. **92**: 967, 1933.
v. Bezold. Untersuchungen über die Innervation des Herzens, 221, 276, Leipsig, 1863.
v. Bezold and Bensen. Neue Wurzburger Zeitung, 1866.
Biasotti. Rev. de la Soc. Argentina d. Biol. **3**: 279, 1927.
Biedl. Pflüger's Arch. **67**: 453, 1897.
Bikeles. Centralbl. f. Physiol. **12**: 346, 1899.
Bikeles and Zbyszewski. Centralbl. f. Physiol. **32**: 277, 1917.
Binet and Gayet. C. R. Soc. Biol. **100**: 177, 180, 338, 1929.
Bochefontaine. Arch. de Physiol. **8**: 140, 1876.
Bochefontaine and Bouceret. C. R. Acad. Sci. **85**: 1168, 1877.
Bonuzzi. Med. Jahrb. 473, 1885.
Boothby. Am. J. Physiol. **14**: 330, 1913.
Bornezzi. Abst. in Centralb. f. Physiol. 801, 1887.

BOSHAMER. Pflüger's Arch. **209**: 784, 1925.
BOWDITCH AND WARREN. J. Physiol. **7**: 416, 1886.
BOWEN, COOMBS AND PIKE. Proc. Soc. Exp. Biol. and Med. **19**: 181, 1922.
BRACHET. Recherches exper. sur les fonctions du systeme nerveux ganglionaire. Paris, 1830.
BRADFORD. J. Physiol. **10**: 358, 1889.
BRADFORD AND BAYLISS. J. Physiol. **16**: 10, 1894.
BRADFORD AND DEAN. J. Physiol. **10**: Proc. 1, 1889.
J. Physiol. **16**: 34, 1894.
Proc. Roy. Soc. B. **45**: 369, 1888.
BRESLAUER. Deutsch. Ztschr. f. Chir. **150**: 50, 1919.
BROWN AND ADSON. Arch. Neur. and Psych. **38**: 323. 1929.
BROWN-SEQUARD. Med. Exam. Phil. August, 490, 1852.
BROWN-SEQUARD AND THOLOZAN. J. de Physiol. **1**: 500, 1858.
BRUCE. Arch. exp. Path. u. Pharm. **63**: 425, 1910.
Quart. J. Exp. Physiol. **6**: 339, 1913.
v. BRÜCKE. Ztschr. f. Biol. **67**: 507, 1917.
v. BRÜCKE. J. Comp. Neur. **53**: 225, 1931.
BRUNTON AND TUNNICLIFFE. J. Physiol. **17**: 272, 364, 1894.
BULGAK. Virchow's Arch. **69**: 162, 1877.
BUNCH. J. Physiol. **22**: Proc. 58, 1898.
J. Physiol. **24**: 72, 1899.
BURN. J. Physiol. **56**: 232, 1923.
J. Physiol. **60**: 365, 1925.
J. Physiol. **75**: 144, 1932.
J. Physiol. **81**: Proc., 1934.
BURN AND DALE. J. Physiol. **61**: 185, 1926.
BURTON OPITZ. Am. J. Physiol. **9**: 161, 1902.
Pflüger's Arch. **123**: 553, 1908.
Pflüger's Arch. **124**: 469, 1908.
Pflüger's Arch. **129**: 189, 1909.
Pflüger's Arch. **135**: 205, 1910.
Quart. J. Exp. Physiol. **5**: 189, 1912.
Pflüger's Arch. **146**: 344, 1912.
Quart. J. Exp. Physiol. **7**: 57, 1913.
Am. J. Physiol. **41**: 103, 1916.
Am. J. Physiol. **42**: 498, 1916–1917.
BURTON OPITZ. Am. J. Physiol. **45**: 64, 1917–1918.
BURTON OPITZ AND EDWARDS. Am. J. Physiol. **43**: 408, 1917.
BURTON OPITZ AND LUCAS. Proc. Soc. Exp. Biol. **6**: 73, 1909.
Pflüger's Arch. **127**: 143, 1909.
BUSQUET. C. R. Soc. Biol. **74**: 891, 1914.
J. de Physiol. **17**: 9, 1917.
CANNON, QUERIDO, BRITTON AND BRIGHT. Am. J. Physiol. **79**: 466, 1927.
CANNON. Am. J. Physiol. **97**: 592, 1931.
CARLSON. Am. J. Physiol. **19**: 408, 1907.
CARLSON. Am. J. Physiol. **31**: 318, 1913.
CARNOT, GAYET AND MERKLIN. C. R. Soc. Biol. **104**: 1263, 1930.

CARRIER. Am. J. Physiol. **61**: 529, 1922.
CATHCART AND CLARK. J. Physiol. **49**: 301, 1915.
CAVANI. Arch. ital. de Biol. **39**: 1903.
CAVAZZANI AND MANCA. Arch. ital. de Biol. **24**: 33, 295, 1895.
CHAUCHARD. C. R. Soc. Biol. **92**: 577, 1925.
CHAUVEAU. Quoted by DASTRE AND MORAT.
COHEN AND BOTHMAN. Am. J. Physiol. **81**: 665, 1927.
COLLIER, DENSHAM AND WELLS. Quart. J. Exp. Physiol. **18**: 291, 1927.
CONHEIM AND ROY. Virchow's Arch. **92**: 436, 1883.
CONTY AND CHARPENTER. C. R. Acad. Sci. **85**: 161, 1877.
CORBEILLE. Am. J. Physiol. **91**: 499, 1929–30.
CORBEILLE, CORBEILLE AND BALDES. Am. J. Physiol. **91**: 505, 1929–30.
COSSY. Arch. de Physiol. **8**: 832, 1876.
COTTON, SLADE AND LEWIS. Heart **6**: 227, 1917.
COURTADE AND GUYON. C. R. Soc. Biol. **53**: 335, 1901.
CRAMER. J. Physiol. **50**: Proc. 38, 1916.
CRAMER. Fever, heat regulation, climate, and the thyroid adrenal apparatus. Longmans Green. 1928.
CROWDEN. J. Physiol. **68**: 313, 1929-30.
CROWDEN AND PEARSON. J. Physiol. **65**: Proc. 25, 1928.
CRUIKSHANK. Quoted by TIGERSTEDT.
CYON. Mélanges Biologiques de l'Acad. de St. Petersbourg **7**: 762, 1871.
C. R. Acad. Sci. **123**: 167, 1896.
C. R. Acad. Sci. **124**: 154, 1897.
Pflüger's Arch. **72**: 370, 1898.
Pflüger's Arch. **70**: 126, 1898.
DALE. J. Physiol. **34**: 163, 1906.
J. Physiol. **46**: 291, 1913.
J. Physiol. **80**: Proc. 10, 1934.
Linacre Lecture. Brit. Med. J. 1934b.
DALE AND EVANS. J. Physiol. **56**: 125, 1922.
DALE AND GADDUM. J. Physiol. **70**: 109, 1930.
DALE AND LAIDLAW. J. Physiol. **52**: 355, 1918.
DALE AND RICHARDS. J. Physiol. **52**: 110, 1918.
DALE AND RICHARDS. J. Physiol. **63**: 201, 1927.
DANIELOPOLU, MARCU, PROCA AND ASLAM. Presse Méd. **26**: 479, 1932.
DASTRE AND MORAT. Various papers in C. R. Acad. Sci. 1880 onwards.
Arch. d. Physiol. **14B**: 326, 1882.
Systeme nerveux vasomoteur. Paris, 1884.
DAVIS. Quoted by RANSON, Physiol. Rev. **1**: 477, 1921.
Am. J. Physiol. **59**: 381, 1922.
DELEZENNE. Arch. de Physiol. **26**: 170, 315, 1895.
DENNIG. Klin. Wchnschr. **31**: 727, 1924.
DENSHAM AND WELLS. Quart. J. Exp. Physiol. **18**: 175, 1927.
DIMITRENKO. Dissertation. Odessa, 1916.
DITTMAR. Arbeiten aus dem Physiol. Anstalt., Leipsig, 55 seq., 1870.
Ber. Sachs Ges. math. Phys. **18**: 1870; 449, 1873.

DOGIEL. Pflüger's Arch. **5**: 136, 1871.
Arch. f. Anat. u. Physiol., Physiol. Ab. 422, 1880.
DOI. J. Physiol. **54**: 227, 1920.
DONEGAN. J. Physiol. **55**: 226, 1921.
DOYON. Arch. de Physiol. **22**: 774, 1890.
DRESCHFIELD. Untersuch. aus dem Physiol. Lab. Wurzburg, 1867.
DRINKER AND DRINKER. Am. J. Physiol. **40**: 514, 1916.
DRURY, FLOREY AND FLOREY. J. Physiol. **68**: 173, 1929.
DUNLOP. J. Physiol. **67**: 349, 1929.
DUNLOP AND WATERSTON. Work at present proceeding in King's College, University of London, 1934.
DUPUY. Quoted by VULPIAN.
DZIEDZIUL. Quoted in Hoffmann & Schabb's Jahresb. **9**: 67, 1880.
EBBECKE. Pflüger's Arch. **169**: 1, 1917.
ECKHARD. Beitr. Anat. u. Physiol. **3**: 123; **3**: 140; **3**: 153; **3**: 150; 1863.
Beitr. z. Anat. u. Physiol. **7**: 75, 1873; **10**: 1, 1883.
EDGREN. Nord. Med. Arch. **37** des. S. A., 1880.
Nordisk. Med. Arkiv. **60** des S. A., 1880.
EDMUNDS. J. Physiol. **22**: 380, 1898.
ELLIS. J. Physiol. **6**: 437, 1885.
FELDBERG. J. Physiol. **61**: 518, 1926.
FELDBERG AND SCHIFF. Pflüger's Arch. **212**: 365, 1926.
FERRIS AND HARVEY. Proc. Soc. Exp. Biol. Med. **22**: 383, 1925.
FLEISCH. Pflüger's Arch. **225**: 26; **226**: 393, 1930.
Pflüger's Arch. **171**: 128, 1918.
Ztschr. f. allg. Physiol. **19**: 269, 1921.
Pflüger's Arch. **226**: 393, 399, 1931.
FLETCHER. J. Physiol. **22**: 259, 1898.
FLOREY, MARVIN AND DRURY. J. Physiol. **65**: 204, 1928.
FOFANOW AND TSCHALUSSOW. Pflüger's Arch. **151**: 543, 1913.
FORBES, SMITH, LAMBERT, CAVENESS AND DERBYSHIRE. Am. J. Physiol. **103**: 131, 1933.
FOSTER. Textbook of physiology, 6th ed., 345, 1893.
FRANÇOIS-FRANCK. Arch. de Physiol. 551, J. de Physiol. 730, 1899.
J. de Physiol. 1205, 1899.
J. de Physiol. **1**: 724, 753, 1899.
Arch. d. Physiol. **22**: 129, 1890.
Arch. d. Physiol. 729, 1893.
Arch. de Physiol. **19**: 122–138, 744–816, 1895.
J. de Physiol., 1210, 1899.
FRANÇOIS-FRANCK AND HALLION. Arch. de Physiol. **29**: 661, 1897.
Arch. de Physiol. 671, 1897.
Arch. de Physiol. **28**: 478, 1896.
Arch. de Physiol. **28**: 908, 923, 1896.
Arch. de Physiol. 661, 1897.
Arch. de Physiol. **28**: 434, 448, 1896.
J. de Physiol. 441, 1908.
FRANÇOIS-FRANCK. C. R. Soc. Biol. **1**: 963, 1909.

FREY. Arbeit. a. d. Physiol. Anstalt Leipsig. (Ludwig's) **11**: 89, 1876.
GAISBOCH. Ber über die Sitzung d. Wiss Arztesges., Innsbrück, 1928.
GANZ. Pflüger's Arch. **3**: 8, 1870.
GASK AND ROSS. Surgery of the Sympathetic Nervous System. London 1934.
GASKELL. J. Anat. and Physiol. **11**: 360, 720, 1877.
J. Physiol. **1**: 108, 262, 1878.
J. Physiol. **7**: 1, 1886.
The involuntary nervous system, Longmans, 1916.
GASSER AND LOEVENHART. J. Pharm. Exper. Ther. **5**: 239, 1913–14.
GERGENS AND WERBER. Pflüger's Arch. **13**: 44, 1876.
GESELL. Am. J. Physiol. **47**: 438, 1919.
GILDING. J. Physiol. **74**: 34, 1932.
GOLLWITZER-MEIER AND SCHULTE. Pflüger's Arch. **229, 251, 264**: 1931.
Arch. f. Exp. Path. u. Pharmacol. **165**: 685, 1932.
GOLTZ. Versamml. d. deutsch. Naturforscher in Königsberg, quoted by T. 278, 1860.
Virchow's Arch. **29**: 394, 1864a.
Zentralbl. f.d. Med. Wiss. 625, 1864b.
Pflüger's Arch. **9**: 189, 1874.
Pflüger's Arch. **8**: 460, 1874.
Pflüger's Arch. **11**: 52, 1875; **14**: 412, 1877.
GOLTZ AND EWALD. Pflüger's Arch. **63**: 362, 1896.
GOLTZ AND FREUSBERG. Pflüger's Arch. **9**: 174, 1874.
GOLTZ, FREUSBERG AND GERGENS. Pflüger's Arch. **11**: 52, 1875.
GOORMAGHTEGH. Arch. Biol. Paris **41**: 109, 1931.
GRANT. Heart **15**: 257, 1929–31.
GRANT AND BLAND. Heart **15**: 385, 1931.
GRANT, BLAND AND CAMP. Heart **16**: 19, 1932.
GRAY. Quart. J. Med. **5**: 300, 1912.
GRIFFITHS AND EMERY. Am. J. Physiol. **95**: 20, 1930.
GROSSMAN. Ztschr. f. Klin. Med. **32**: 1, 1897.
GRUBER. Am. J. Physiol. **37**: 259, 1915.
Am. J. Physiol. **40**: 136, 1916.
Am. J. Physiol. **42**: 214, 1917.
GRUBER AND KRETSCHNER. Am. J. Physiol. **46**: 222, 1918.
GRÜTZNER AND HEIDENHAIN. Pflüger's Arch. **16**: 1, 42, 1877.
HALES. Statistical essays, vol. 2, 1733.
HALLION AND COMTE. Arch. de Physiol. **26**: 381, 1894.
C. R. Soc. Biol. **51**: 977, 1899.
HALLION AND MOREL. J. de Physiol. **14**: 1, 1912.
HAMET. C. R. Soc. Biol. **109**: 1357, 1932.
HARGIS AND MANN. Am. J. Physiol. **68**: Proc. 116, 1924.
HARRIS AND MARVIN. Heart **14**: 135, 1927.
HARROP, REHBERG AND KROGH. J. Physiol. **56**: 179, 1922.
HARTMAN, BLATZ AND KILBORN. J. Physiol. **53**: 92, 1919.
HASAMA. Arch. int. Pharmacol. **41**: 145, 1931.
HASTERLIK AND BIEDL. Wien. Klin. Wchnschr. **6**: 43, 1893.
HEAD AND BAYLISS. Quoted by BAYLISS, The vasomotor system, p. 31.

HEDON. Travaux de Physiol., Paris, 263.
C. R. Soc. Biol. **1**: 952, 1906.
HEGER. Beitr. z. Physiol. (Ludwig's), 193, 1887.
HEIDENHAIN, LANDAU AND PACULLY. Pflüger's Arch. **2**: 423, 1869.
HEIDENHAIN. Pflüger's Arch. **3**: 504, 1870.
Pflüger's Arch. **5**: 77, 309, 1872.
HEIDENHAIN AND GRÜTZNER. Pflüger's Arch. **16**: 1, 1878.
HEMINGWAY AND MCDOWALL. J. Physiol. **62**: 166, 1926.
HENDERSON. Am. J. Physiol. **19**: Proc. 14, 1907.
Am. J. Physiol. **21**: 126, 1908.
Am. J. Physiol. **24**: 66, 1909.
J. A. M. A. **74**: 783, 1920.
HENDERSON AND HAGGARD. J. Biol. Chem. **33**: 355, 1918.
HENDERSON, BARRINGER AND HARVEY. Am. J. Physiol. **23**: Proc. 30, 1909.
HENDERSON AND HARVEY. Am. J. Physiol. **46**: 533, 1918.
HENLEY. Allgemeine Anatomie. Leipsig, 525, 690, 1841.
HERING. Sitz. d. k. Akad. d. Wiss. Wien. **60**: 839, 1869.
Der. Blutdruckzüglertonus etc., Thieme, Leipzig, 5, 1932.
HERRICK, ESSEX AND BALDES. Am. J. Physiol. **101**: 213, 1932.
HERRICK, ESSEX AND BALDES. Am. J. Physiol. **103**: 592, 1933.
HESS. Die Regulierung des Blutkreislaufes, Theime, Leipsig, 1930.
HEYMANS. Le Sinus carotidien et la zone homologue cardioaortic, Doin, Paris, 1933.
C. R. Soc. Biol. **99**: 1236, 1928.
HEYMANS AND BOUCKAERT. C. R. Soc. Biol. **100**: 104, 202, 1929.
C. R. Soc. Biol. **111**: 145, 1932.
C. R. Soc. Biol. **110**: 996, 1932.
HEYMANS, BOUCKAERT AND DAUTREBANDE. C. R. Soc. Biol. **105**: 217, 1930.
C. R. Soc. Biol. **106**: 48; Arch. Intern. Pharm. Therap. **40**: 292, 1931.
HILL, L. J. Physiol. **18**: 15, 1895.
J. Physiol. **22**: Proc. 26, 1898.
HILL AND FLACK. J. Physiol. **37**: 77, 1908.
J. Physiol. **40**: 360, 1910.
HINSEY AND GASSER. Am. J. Physiol. **92**: 679, 1930.
HIRCHFELD. Veroffentl. d. Zentral J. Balneologie, 1912.
HOCHREIN AND GROS. Pflüger's Arch. **229**: 642, 1932.
HOCHREIN AND KELLER. 1932.
HOLTMEIER. Münch. Med. Wchnschr. **16**: 682, 1927.
HOOKER, D. R. Am. J. Physiol. **28**: 361, 1911.
Am. J. Physiol. **31**: 47, 1912.
Am. J. Physiol. **46**: 591, 1918a.
Am. J. Physiol. **45**: Proc. 543, 1918b.
Am. J. Physiol. **54**: 30, 1920.
Physiol. Rev. **1**: 1, 1921.
HOOKER, WILSON AND CONNETT. Am. J. Physiol. **43**: 351, 1917.
HORIUCHI. Pflüger's Arch. **206**: 473, 1924.
HOUGH AND BALLANTYNE. J. Med. Sci. Boston **3**: 330, 1899.
HOWELL AND AUSTIN. Am. J. Physiol. **3**: Proc. 22, 1900.

Howell, Budgett and Leonard. J. Physiol. **16**: 298, 1894.
Hunt. J. Physiol. **18**: 381, 1895.
Huisey. J. Comp. Neurol. **47**: 23, 1928.
Huizinga. Pflüger's Arch. **11**: 207, 1875.
Isergin. Arch. f. Anat. u. Physiol. 440, 1894.
Januschka. Wien. Klin. Wchnschr. **26**: 869, 1913.
Japelli and Tria. Quoted in Jahresb. Physiol., 1907.
Jarisch. J. Physiol. **60**: 419, 1926.
Jarish and Ludwig. Arch. f. exp. Path. u. Pharmacol. **114**: 240, 1926.
Johansson. Bihang Lill. K. Svenka Vet.-akad. hand. 16 afd. 4, nr. 4, p. 37, 1890.
Kahn. Centralbl. f. Physiol., 1904.
Kabierski. Pflüger's Arch. **14**: 518, 1877.
Karlik. Pflüger's Arch. **222**: 356, 1929.
Karpus and Kreidl. Pflüger's Arch. **171**: 192, 1918.
Pflüger's Arch. **215**: 667, 1927.
Kaufmann. Pflüger's Arch. **147**: 46, 1912.
Kaya and Starling. J. Physiol. **39**: 346, 1909–10.
Kendall and Luchsinger. Pflüger's Arch. **13**: 197, 1876.
Ken Kure. Quart. J. Exp. Physiol. **21, 22**: 1930–31.
Kerper and Collier. Proc. Soc. Exp. Biol. and Med. **24**: 493, 1927.
Kibjakow. Pflüger's Arch. **228**: 30, 1931.
Klemensrewiez. Sitzungsb. d. Wien. Akad. d. Wiss. m/n k/l 94(3), 78, 1886.
Kleen. Skand. Arch. f. Physiol. **1**: 247, 1889.
Knoll. Sitzungsb. d. Akad. d. Wiss. zu Wien. m/n cl. 92 Abs. 3, 449, 1885.
Koch. Die reflectoriche Selbststeuerung des Kreislaufes, Steinkoff., Dresden, Leipsig, 1931.
Koch and Nordmann. Ztschr. f. Kreislauf. **20**: 343; Ztschr. Exp. Med. **7**: 241, 1928.
Koch and Mark. Ztschr. f. Kreislauf. **23**: 319, 1928.
Koch, Hochrein and Keller. Arch. f. exp. Path. u. Pharmacol. **160**: 205, 1931.
Koch and Simon. Klin. Wchnschr. **7**: 2104, 1928.
Koenigs. J. de Physiol. 721, 1912.
Kolm and Pick. Arch. f. exp. Path. **87**: 135, 1920.
Konow and Stenbeck. Skand. Arch. f. Physiol. **1**: 403, 1889.
Kowelewsky and Adamuk. Cent. f. d. med. Wiss. 525, 529, 1868.
Kremer and Wright. Quart. J. Exp. Physiol. **21**: 319, 1932.
Krimer. Physiol. Untersuch. Leipsig, 1820.
Krogh. J. Physiol. **52**: 409, 1919a.
J. Physiol. **52**: 457, 1919b.
J. Physiol. **53**: Proc. 47, 1919c.
J. Physiol. **53**: 399, 1920.
Danske Videns. Kabenes Selskab Biologiske Meddelelsier 3, Nr. 3, 14, 1921.
J. Physiol. **55**: 412, 1921.
The anatomy and physiology of the capillaries. 2nd ed., Yale Univ. Press, 1929.
Krogh and Brandt-Rehberg. J. Physiol. **64**: Proc. 32, 1927.
Krogh, Harrop and Rehberg. J. Physiol. **56**: 179, 1922.

KUCKSWELLER. Eckhard's Beitr. z. Anat. u. Physiol. **11**: 215, 1885.
LACEY. Proc. Soc. Exp. Biol. **29**: 1074, 1932.
LAFFONT. C. R. Acad. Sci. **89**: 649, 1879.
C. R. Acad. Sci. **90**: 705, 1880.
LAFFONT AND VITZORE. C. R. Soc. Biol. 310, 1879.
LANCELIN. C. R. Soc. Biol. **80**: 160, 1917.
LANGLEY. J. Physiol. **1**: 96, 1878.
J. Physiol. **12**: 375, 1891.
J. Physiol. **14**: Proc. 2, 1893.
J. Physiol. **17**: 296, 1894.
Phil. Trans. Roy. Soc. **183**: 85, 1892.
J. Physiol. **20**: 223, 1896.
J. Physiol. **41**: 483, 1911.
J. Physiol. **45**: 239, 1912.
J. Physiol. **53**: 147, 1919.
The autonomic nervous system. Heffer, Cambridge, 1921.
J. Physiol. **58**: 49, 70, 1923.
J. Physiol. **59**: 231, 1924.
J. Physiol. **60**: Proc. 17, 1925.
LANGLEY AND ANDERSON. J. Physiol. **16**: 410, 1894.
J. Physiol. **18**: 67, 1895.
J. Physiol. **19**: 71, 1895–96.
LATCHENBERGER AND DEAHNA. Pflüger's Arch. **12**: 157, 1876.
Pflüger's Arch. **12**: 189, 1876.
LEFEVRE. C. R. Soc. Biol. **15**: 80, 1899.
LEHNDORFF. Arch. f. Anat. u. Physiol. 362, 1908.
LEPINE. C. R. Soc. Biol. 65, 1876.
LERICHE AND HEITZ. C. R. Soc. Biol. **160**: 415, 1917.
LERICH AND POLICARD. C. R. Soc. Biol., 415, 1920.
LEWASCHEW. Referred to Jahrb. Anat. Physiol. **2**: 47, 1884.
LEWIN AND SCHILF. Pflüger's Arch. **216**: 657, 1927.
LEWIS. The blood vessels of the human skin. Shaw, London, 1927.
J. Physiol. **64**: Proc. 36, 1928.
Heart **15**: 177, 1930.
Brit. Med. J. **1**: 873, 1932.
LEWIS AND GRANT. Heart **11**: 209, 1924.
LEWIS, GRANT AND MARVIN. Heart **14**: 139, 1927.
LEWIS AND PICKERING. Heart **16**: 33, 1931.
LEWIS AND ZOTTERMAN. Heart **13**: 203, 1926.
LIDDELL AND SHERRINGTON. Mammalian physiology. Oxford Med. Press, 1929.
LISTER. Phil. Trans. Roy. Soc. P. **2**: 607, 1858.
LOEB. Beitr. z. Anat. Physiol. Giessen **5**: 86, 1870.
LOVÉN. Ber. d. Sachs Ges. d. Wiss., math./phys. k/l 89, Leipsig, 1866.
LUCHSINGER. Pflüger's Arch. **14**: 391, 1877.
Pflüger's Arch. **16**: 518, 1878.
LUDWIG. Arch. exp. Path. u. Pharmacol. **160**: 302, 1931.
LUDWIG AND CYON. Ber. über die verh. d. Konigslich Sachs ges d. Wiss. math. phys. kl. 18, 307, 1866.

LUDWIG AND THIRY. Sitzungsb. Akad. Wiss. Math.-Nat. Wien. **49**: 2, 421, 1864.
McDOWALL. J. Physiol. **55**: Proc. 1, 1921.
J. Physiol. **56**: Proc. 44, 1922.
J. Physiol. **59**: 41, 1924.
J. Pharmacol Exp. Therap. **25**: 289, 1925.
J. Physiol. **61**: 131, 1926.
J. Physiol. **69**: Proc. 9, 11, 1930.
J. Physiol. **70**: 301, 1930.
J. Physiol. **71**: 417, 1931.
Quart. J. Exp. Physiol. **23**: 269, 1933.
Brit. Med. J. **1**: 690, 1933.
Quart. J. Exp. Physiol. **23**: 277, 1933.
Brit. Assn. Rept. 535, 1933.
J. Physiol. **81**: Proc. 5, 1934.
McDOWALL AND WELLS. Nature, October, 1926.
McLEAN. Am. J. Physiol. **22**: 279, 1908.
McSWINEY. Physiol. Rev. **11**: 478, 1931.
MAGITOT AND BAILLART. J. de Physiol. **19**: 534, 1921.
MAJENDIE. Quoted by VULPIAN AND TIGERSTEDT.
MALL. Arch. f. Anat. and Physiol. 1892, 420.
Arch. f. Anat. u. Physiol., sup. 57, 1890.
MARAGLIANO AND LUSONA. Arch. ital. d. Biol. **11**: 246, 1889.
MARCU. J. Physiol. **67**: Proc. 36, 1929.
MARES. Pflüger's Arch. **91**: 529, 1902.
Pflüger's Arch. **97**: 567, 1903.
MAREY. Annale de Sci. nat. 4 ser. Zoolog. **9**: 68, 1858. (See also Vulpian, Leçons sur l'appareil vasomotor, 1, 46.)
MARTIN. Am. J. Physiol. **59**: 400, 1922.
MARTIN, FRANKLIN AND HIELD. Am. J. Physiol. **53**: 421, 1920.
MARTIN AND LACEY. Am. J. Physiol. **33**: 212, 1914.
MARTIN AND MENDENHALL. Am. J. Physiol. **38**: 98, 1915.
MARTIN AND STILES. Am. J. Physiol. **34**: 106, 220, 1914.
Am. J. Physiol. **37**: 94, 1915.
Am. J. Physiol. **40**: 194, 1916.
MARTIN AND JACOBY. Am. J. Physiol. **59**: 394, 1922.
MARTIN AND WHITE. Am. J. Physiol. **60**: 323, 1922.
MASSUIS AND VANLAIR. C. R. Internat. Cong. Med. Brussels, 28 of S. A., 1875.
MATHISON. J. Physiol. **41**: 416, 1910.
J. Physiol. **42**: 283, 1911.
MAXIMOWITCH. Deutsch. Arch. f. klin. Med. **56**: 440, 1896.
MAYER AND PRIBRAM. Sitzungsb. Akad. Wiss. math. natur. Wien. **66** (3), 102, 1872.
METALLINOS. Dissertation Berlin, quoted by SCHILF, 1925.
MILLER. Pflüger's Arch. **43**: 1, 1912.
MISLAWSKY AND BISTRENIN. J. de Physiol. **7**: 1002, 1905.
MISLAWSKY. Arch. Internat. de Physiol. **3**: 381, 1906.
MOISSEJEFF. Ztschr. f. exp. Med. **53**: 696, 1926.
MORAT. Arch. de Physiol. **24**: 689, 1892.

MORAT AND DOYON. Arch. d. Physiol. **24**: 60, 1892.
MOREAU. Memoires de Physiol., Paris, 1877.
MOSONYI. Pflüger's Arch. **217**: 131, 1927.
MOSSO. Ber. d. Sachs Ges. d. Wiss. m/p, 1874.
Fatigue. English transl., 5th ed., Longmans, 1923.
MÜLLER. Deutsch. Arch. f. klin. Med. **105**: S. A. 10, 1905.
Deutsch. Ztschr. J. Nervenheilk. **47**: 417, 1913.
MUNK. Ztschr. f. exp. Path. **8**: 337, 1910.
NATHUN. Quoted by HENDERSON, PRINCE AND HAGGARD. J. Pharmacol **41**: 203, 1918.
NICOLAIDES. Arch. f. Anat. u Physiol. 28, 1882.
NORDMAN AND MÜLLER. Klin. Wchnschr. **11**: 1371, 1932.
NORLIN. Skand. Arch. f. Physiol. **64**: 239, 1932.
OGATA AND VINCENT. J. Comp. Neurol. **30**: 355, 1919.
OINUMA. J. Physiol. **43**: 343, 1911. See LANGLEY: J. Physiol. **41**: 494, 1911.
Ann. de Physiol. a. d. Physic. Chem. Biol. **1**: 1, 1925.
ONIMUS AND LEGROS. Thesis, Paris, 1872.
OSSOKIN. Ztschr. f. Biol. **63**: 443, 1914.
OSTROUMOFF. Pflüger's Arch. **12**: 219, 1876.
OTT. J. Physiol. **2**: 42, 1879.
OWSJANNIKOFF. Ber. d. k. Sachs de Wiss. Math.-Phys. Cl., Leipsig, 135, 1871.
PAGANO. Arch. ital. d. Biol. 331, 1900.
PAL. Med. Jarhb 67, 1888.
PAPILLIAN AND CRUECANII. C. R. Soc. Biol. **90**: 1063, 1924.
PARSONS. J. Physiol. **29**: Proc. 12, 1903.
PATRIZI. Quoted from Centralbl. f. Physiol. **11**: 604, 1897.
PAVLOV. Pflüger's Arch. **16**: 266, 1878.
Pflüger's Arch. **20**: 210, 1879.
PEARCE. Ztschr. f. Biol. **62**: 243, 1913. See Doi. J. Physiol. **54**: 238, 1920.
Am. J. Physiol. **35**: 151, 1914.
PETROFF. Ztschr. ges. Med. **79**: 506, 1931.
PICK. Arch. f. Anat. u. Physiol., 563, 1872.
PICKERING. Heart **16**: 115, 1932.
PIKE, GUTHRIE AND STEWART. Am. J. Physiol. **21**: 359, 1908.
PIKE. Am. J. Physiol. **24**: 124, 1909.
Am. J. Physiol. **30**: 436, 1912.
PILCHER AND SOLLMANN. Am. J. Physiol. **35**: 59, 70, 1914.
PIOTROWSKI. Centralbl. f. Physiol. 454, 1887.
Centralbl. f. Physiol. 464, 1892.
Pflüger's Arch. **55**: 288, 1894.
PLAVEC. Pflüger's Arch. **79**: 195, 1900.
PORTER AND BEYER. Am. J. Physiol. **4**: 283, 1900.
PORTER AND MUHBERG. Am. J. Physiol. **4**: 334, 1900.
PORTER AND STOREY. Am. J. Physiol. **18**: 181, 1907.
PORTER, MARKS AND SWIFT. Am. J. Physiol. **20**: 444, 1907–08.
PORTER AND MARKS. Am. J. Physiol. **21**: 460, 1908.
PORTER AND RICHARDSON. Am. J. Physiol. **23**: 131, 1908–09.
PORTER. Am. J. Physiol. **27**: 276, 1910.
Am. J. Physiol. **36**: 418, 1915.

PORTER AND TURNER. Am. J. Physiol. **39**: 236, 1915.
POUSSACK. Ber. d. Sachs Ges. d. Wiss. m/p 116, 1868.
PREOBRASCHENSKY. Quoted in Jahresb. d. Physiol. 67, 105, 1892.
PRUSSAK. Ber. d. Sachs Ges. d. Wiss. Mat. Phys. 117, 1868.
PUELMA AND LUCHSINGER. Pflüger's Arch. **18**: 489, 1878.
PUTZEYS AND TARCHANOFF. Reichert u. DuBois Reymond's Arch. 371, 1874.
RAJKA AND FURTH. Deutsch. med. Wchnschr. **1**: 6, 1924.
RANKE. Die Blutwertheilung und der Tätigkeitswechsel der Organ. Leipsig, 69, 1871.
RANSON. Physiol. Rev. **1**: 447, 1921.
RANSON AND BILLINGSLEY. Am. J. Physiol. **41**: 85, 1916.
Am. J. Physiol. **42**: 1, 9, 1916.
RANSON AND HESS. Am. J. Physiol. **38**: 98, 1915.
RANSON AND WIGHTMAN. Am. J. Physiol. **62**: 382, 405, 1922.
REED. Am. J. Physiol. **74**: 61, 1925.
REID. Quoted by TIGERSTEDT.
REID HUNT. J. Physiol. **18**: 381, 1895.
REIN. Klin. Wchnschr. **2**: 1485, 1930.
Ztschr. f. Biol. **89**: 307, 1931.
Ergebn. d. Physiol. **32**: 28, 1931.
REISSNER. Arch. f. Anat. u. Physiol. 125, 1862.
RELGER AND FORSTER. Arch. f. Ophthal. 7 (3): 146, 1881.
RICHET AND DUBLINEAV. C. R. Soc. Biol. **105**: 537, 1930.
RICHARDSON AND WYATT. Am. J. Physiol. **45**: Proc. 555, 1918.
ROEVER. Quoted in HEYMANS' Monograph, 1869.
ROGERS. C. R. Soc. Biol. **222**: 646, 1890.
ROSENBLUETH AND CANNON. Am. J. Physiol. **108**: 599, 1934.
ROUGET. J. Anat. nom. et Path. **5**: 556, 1879.
ROY AND ADAMI. Phil. Trans. Roy. Soc. B. **183**: 199, 1892.
ROY AND GRAHAM BROWN. J. Physiol. **2**: 338, 1879.
RUTHERFORD. J. Anat. and Physiol. **3**: 402, 1869.
SADLER. Ber. d. Sachs Ges. d. Wiss. math./phy. kl., 195, 1869.
SAMUEL. Virchow's Arch. **121**: 396, 1900.
SASKI. Ztschr. f. Klin. Med. **71**: 456, 1910.
SCHÄFER AND MOORE. J. Physiol. **20**: 1, 1896.
SHARPEY-SCHAFER. Quart. J. Exp. Physiol. **12**: 231, 1920; J. Physiol. **53**: Proc. 23, 1919.
Quart. J. Exp. Physiol. **12**: 381, 1920.
SCHIFF. Untersuch. z. Physiol. d. Nervensystem, Frankfurt-a-M. **80**: 197, 1855.
Berner Schriften, p. 69, 1856.
Untersuch. über Zuckerbildung in der Leber. Wurzburg, 153, 1859.
SCHILF. Klin Woch. **3**: 414, 1924.
SCHILF. Das autonome Nerven system. Thieme, Leipsig, 1926.
SCHLESINGER. Med. Jahr. 20, 1874.
SCHMIDT. Pflüger's Arch. **126**: 165, 1909.
SCHOLER. Inaug. Diss. Dorpat, 42, 1869.
SCHULTEN. Arch. f. Ophthal. **30** abs. 3, 47, 1884.
SCOTT AND ROBERTS. J. Physiol. **58**: 168, 1923.
SCOTT. J. Physiol. **59**: 443, 1924.

SEELIG AND LYON. J. Am. Med. Assoc. **52**: 45, 1909.
SEPPÄ. Skand. Arch. f. Physiol. **38**: 49, 1919.
SERGENT. Press. méd. Paris **11**: 813, 1903.
SEWALL AND SANBFORD. J. Physiol. **11**: 179, 1890.
SEWALL AND STEINER. J. Physiol. **6**: 162, 1885.
SHERRINGTON. Integrative action of the nervous system. 1906.
J. Physiol. **38**: 375, 1909.
SIMON. Ztschr. f. Kreislauf. **21**: 448, 1929.
SINAKEWITCH. Arch. internat. de Physiol. **4**: 51, 1906.
SMIRNOW. Centralbl. med. Wiss. **24**: 146, 1886.
Anat. Anz. **10**: 737, 1895.
SNELLEN. Arch. f. g. Holland Beitr. **1**: 213, 1858.
SOLLMANN AND BROWN. Am. J. Physiol. **30**: 102, 1912.
SOLLMANN AND PILCHER. Am. J. Physiol. **26**: 247, 1910.
Am. J. Physiol. **30**: 303, 369, 1912.
SPALLITTO AND CONSIGLIO. Arch. ital. de Biol. **28**: 231, 1897.
SPEISS. Münch. med. Wchnschr. **53**: 345, 1906.
SPERANSKAJA-STEPANOWA. Pflüger's Arch. **210**: 627, 1925.
STAHL. Thèse. Bruxelles, 1883.
Pflüger's Arch. **203**: 57, 1924.
STELLING. Nervus depressor. Inaug. Diss. Dorpat, 1867.
STEPANOW. Skand. Arch. f. Physiol. **38**: 3, 1918.
Proc. Russ. Physiol. Soc., March 11, 1922—quoted from Physiol. Abst.
Proc. Russ. Physiol. Soc., July 22, 1922b—quoted from Physiol. Abst.
Russ. J. Physiol. **4**: 217, 1922.
STEPHANI. Arch. Ital. de Biol. **24**: 424, 1895.
STEVENSON AND REID. Bull. Johns Hopkins Hosp. **26**: 21, 1915.
STEWART. Heart **3**: 77, 1911.
STEWART, GUTHRIE, BURNS AND PIKE. J. Exp. Med. **8**: 289, 1906.
STEWART AND PIKE. Am. J. Physiol. **19**: 341, 1907.
STEWART AND LAFFER. Arch. Int. Med. **11**: 365, 1913.
STEWART AND WALKER. Arch. Int. Med. **11**: 383, 1913.
STRICKER. Wien. Sitz. **74** (111): 173, 1876.
Med. Jahresb. 279, 1877.
Med. Jahresb. 409, 1978.
Med. Jahresb. 1, 1886.
TAWASTJERNA. Studien über den Kreislauf des Winterfrosches. Akad. Abt. Helsingfors, 1916.
TENGWALL. Skand. Arch. Physiol. **6**: 225, 1895.
TIGERSTEDT. Die Physiologie des Kreislaufes. Gruyter, Berlin and Leipsig, 1923.
THAYER AND PAL. Med. Jahresb. **3**: 33, 1888.
THIRY. Cent. f. d. Wiss., 1864.
THOMPSON. Arch. Anat. u. Physiol., 1893.
J. Physiol. **65**: 441, 1928.
THOMPSON AND MACDICK. Am. J. Physiol. **86**: 542, 1928.
TOURNADE. Lyon Med. 30, 1930.
C. R. Soc. Biol. **109**: 879, 1932.

TOURNADE AND MALMEJAC. C. R. Soc. Biol. **105**: 672, 1930.
C. R. Soc. Biol. **112**: 972, 1933a.
C. R. Soc. Biol. **112**: 228, 1933b.
C. R. Soc. Biol. **112**: 1264, 1933c.
C. R. Soc. Biol. **113**: 610, 611, 1933d.
TRAUBE. Centralbl. f. d. Med. Wiss. Berlin, 32, 1864.
Centralbl. f. d. Med. Wiss. Berlin, 881, 1865.
TRAZASKA AND CHRZONEZEWSKY. Virchow's Arch. **157**: 376, 1899.
TSCHALUSSOW. Pflüger's Arch. **151**: 523, 529, 541, 1913.
TSCHVEWSKY. Pflüger's Arch. **97**: 283, 1903.
TSCHIRWINSKY. Zentralbl. f. Physiol. **10**: 65, 1896.
TWEED, CLARK AND EDDINGTON. Vet. Record **11**: 23, 1931.
USTIMOWITCH. Arch. f. Anat. and Physiol. 185, 1887.
VELICH. Pflüger's Arch. **95**: 264, 1903.
VERCAUTEREN. C. R. Soc. Biol. **109**: 563, 1932.
VINCENT. Quart. J. Exp. Physiol. **15**: 319, 1925.
VINCENT AND CAMERON. Quart. J. Exp. Physiol. **9**: 45, 1916.
VINCENT AND CURTIS. J. Physiol. **63**: 151, 1927.
VINCENT AND OGATA. J. Comp. Neurol. **30**: 355, 1918.
VINCENT AND THOMPSON. J. Physiol. **65**: 449, 1928a.
J. Physiol. **65**: 327, 1928b.
J. Physiol. **66**: 307, 1928c.
J. Physiol. **66**: 449, 1928d.
Lancet **1**: 534, 1929.
VULPIAN. C. R. Acad. Sci. **76**: 662, 1873.
C. R. Acad. Sci. **80**: 330, 1875.
Leçons sur l'appareil vasomotor **1**: 154, 159, 1875.
C. R. Acad. Sci. **101**: 982, 1885.
DE WAELE VAN DE VELDE AND BRAEYE. Arch. int. Physiol. **36**: 17, 1933.
DE WAELE AND VAN DE VELDE. C. R. Soc. Biol. **113**: 85, 1933.
Arch. internat. Phys. **36**: 371, 1933.
Arch. internat. Physiol. **36**: 391, 1933.
WALLER. C. R. Acad. Sci. **36**: 378, 1853.
WATTS. Am. J. Physiol. **38**: 361, 1915.
WEBER, E. H. Hildebrant's Handbuch der Anatomie, 4th ed., Part 3, 75, 1831.
WEBER. Pflüger's Arch. 457, 1908.
WEEKERS. Arch. Internat. de Physiol. **3**: 191, 1906.
WEIDHOPF. Brun's Beitr. z. klin. Chir. **130**: 399, 1923.
WEGNER. Arch. f. Ophthal. **12** (2): 10, 1866.
WERTHEIMER. Arch. de Physiol. **25**: 297, 1893.
Arch. de Physiol. **26**: 308, 724, 1894.
WERZILOFF. Centralbl. f. Physiol. **10**: 194, 1896.
WHEELAN. Cleveland Med. J. **16**: 111, 1917 (a review).
WILSON. Quoted by RANSON: Physiol. Rev. **1**: 477, 1921.
WINKLER. Sitz. Kais Akad. Wiss. Wien. m/n Kl. **111**: 3, 1902.
WOOLLARD. Heart **13**: 319, 1926.
WRIGHT. Endocrinol. **6**: 1100, 1922.
WRIGHT. J. Physiol. 66, 387, 1928.
YATES. Am. J. Physiol. **57**: 68, 1921.
ZUNTZ. Pflüger's Arch. **17**: 135, 374, 1878.